UDC 632.35:632.937.1
579.852.11
Acta Agriculturae Serbica, 29 (57), 27–33, 2024 doi: 10.5937/AASer2357027K

Publisher: Faculty of Agronomy Čačak

www.afc.kg.ac.rs

Biocontrol potential of *Bacillus amyloliquefaciens* D5 ARV metabolites

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Received 29 November 2023; Accepted 20 February 2024

A B S T R A C T

Integrated pathogen management incorporates biological control and ecological services of plant growth-promoting bacteria as base components. The biocontrol activity of *Bacillus amyloliquefaciens* D5 ARV toward *Fusarium oxysporum*, *Fusarium graminearum*, *Botrytis cinerea*, and *Macrophomina* sp. was estimated through a confrontation test, and the potential of volatile and non-volatile organic compounds (VOCs). The results of the confrontation test showed 60, 46, 37, and 33% of *F. oxysporum*, *F. graminearum*, *B. cinerea*, and *Macrophomina* sp. growth inhibition, while VOCs effects reached 30%, 47%, 53%, and 0% growth inhibition, respectively. A collection of non-volatile metabolites was made at a stationary phase; afterward, they were sterilized by filtration or autoclaving. Autoclaving caused a significant loss of non-volatile metabolite antifungal activity. GC-MS analysis of VOCs detected the presence of compounds with antifungal and antimicrobial properties such as pentadecanoic acid, and hexanedioic acid, bis(2-ethylhexyl) ester. The multiple antifungal mechanisms revealed in this study are part of the *B. amyloliquefaciens* D5 ARV arsenal and make it a potentially powerful biocontrol agent against selected phytopathogens.

Keywords: *biocontrol,* Bacillus amyloliquefaciens, Botrytis cinerea, Fusarium *spp.,* Macrophomina *sp., VOCs*

И З В О Д

Биолошка контрола и еколошке услуге које пружају бактерије које подстичу раст биљака јесу компоненте које чине темељ интегралног менаџмента биљних болести. Биоконтролна активност *Bacillus amyloliquefaciens* D5 ARV у односу на *Fusarium oxysporum*, *Fusarium graminearum, Botrytis cinerea* и *Macrophomina* sp. утврђена је кроз тест конфронтације и праћење ефеката неиспарљивих и испарљивих органских једињења (VOCs). Резултати конфронтацијског теста су показали инхибицију раста *F. oxysporum*, *F. graminearum*, *B. cinerea* и *Macrophomina* sp. за 60, 46, 37и 33%, док су ефекти присуства испарљивих компоненти инхибиција од 30, 47, 53 и 0%. Неиспарљиви метаболити су прикупљени у стационарној фази раста и коришћени на два начина, након стерилизације филтрацијом или аутоклавирањем. Неиспарљиви бактеријски метаболити су у значајној мери изгубили своју антифунгалну активност након аутоклавирања. GC-MS анализа испарљивих метаболита је утврдила присуство компоненти са антифунгалним и антимикробним својствима попут пентадеканске киселине и бис(2-етилхексил) естар хександиоичне киселине. Ова студија је показала биоконтролни потенцијал *B. amyloliquefaciens* D5 ARV, који је резултат вишеструких антифунгалних механизама, који га чине потенцијално моћним биоконтролним агенсом против одабраних фитопатогена.

Кључне речи: биоконтрола, *Bacillus amyloliquefaciens, Botrytis cinerea, Fusarium* spp., *Macrophomina* sp., испарљива једињења

1. Introduction

New plant diseases are emerging all over the world and can take epidemic proportions in just a few years (Dimopoulou et al., 2021). The rapid emergence and spread, together with pathogen resistance and cutting down on approved conventional pesticides, make efficient control extremely challenging. Those circumstances put the accent on safe, environmentally acceptable, and nature-based alternatives in crop protection. Integrated pathogen management is an ecosystem-tailored approach that emphasizes biological control as a key component, and plant growth-promoting bacteria (PGPB) are among the most important participants of this approach.

PGPB are soil inhabitants capable of stimulating the growth of plants and reducing the risk of disease. Those capable of reducing the growth and development of phytopathogenic fungi are marked as biocontrol agents (BCAs). The wide spectra of mechanisms from competition for nutrients to induced systemic resistance (ISR) are employed by BCAs. Production of antibiotics is a characteristic of numerous BCAs, which, together with bacteriocins, and siderophores, represent the supreme mechanism of potential biocontrol (El-Saadony et al., 2022). Among the primary strategies

used by BCAs are mycoparasitism, the production of hydrolytic enzymes and metabolites that inhibit phytopathogen growth (Haas and Keel, 2003). Among metabolites, the role of volatile organic compounds (VOCs) is especially interesting considering their ability to trigger long-distance physiological processes. VOCs are usually emphasized in the context of the stimulation of plant growth, production of biomass, and ISR to pathogens (Kerečki et al., 2022; Raza et al., 2016).

The fast-growing markets of BCAs are based on the rapid increase in knowledge regarding natural antagonists of plant pathogens and their metabolites as powerful active compounds (Marrone, 2019; Dimopoulou et al., 2021).

Fusarium oxysporum, *F. graminearum, Botrytis cinerea*, and *Macrophomina* sp. are among the most devastating plant pathogens. Even though the majority of the *F. oxysporum* species complex represent saprotrophs, the pathogenic strains are among the 10 top plant pathogens that cause economic loss all over the world (Lamo and Takken, 2020). The most serious diseases caused by *F. oxysporum* are wilt, foot-rot, and root-rot, which pose huge agricultural problems. *F. graminearum* is a pathogen that causes head blight in cereals resulting in a dramatic reduction in their global yield (Jimenez-Quiros et al., 2022). *B. cinerea* is a necrotroph responsible for gray mold diseases in numerous host plants (Roca-Couso et al., 2021) and represents a serious threat during the prior and post-harvest periods. Worldwide, the annual yield loss caused by *B. cinerea* infections is estimated at billions of dollars (Cheung et al., 2020), which illustrates the seriousness of the problem and the urgent need for a solution.

Macrophomina sp. is responsible for stem rot, root rot, seedling blight, and charcoal rot in more than 500 crops and wild species (Rangel-Montoya et al., 2022). *Macrophomina phaseolina*, with its effect on soybean expressed through the development of charcoal rot, is settled at the top of the list of pathogens responsible for global yield decrease (Savary et al., 2019). Only in the United States during a four-year period, this pathogen caused damage that was estimated at 220 billion dollars (Allen et al., 2017).

The list of hosts of these phytopathogenic fungi often overlaps, including some of the most important crops for global food production such as corn, sunflower, cotton, soybean, sorghum, strawberry, etc. (Roca-Couso et al., 2021; Lamo and Takken, 2020; Rangel-Montoya et al., 2022). Those fungi are capable of developing fungicide resistance and causing huge economic consequences (Amselem et al., 2011; Lamo and Takken, 2020).

Approximately 50% of BCAs' fungicides and bactericides available in the European and USA market are based on *Bacillus* spp. (Dimopoulou et al., 2021). Among others, *B. amyloliquefaciens* is recognized as a powerful BCA available in different formulations from powder to concentrated cell and aqueous suspensions. It is registered as a biofungicide against *Fusarium* spp., *Rhizoctonia* spp., and *Aspergillus* spp. (EPA, 1999). Recently, *B. amyloliquefaciens* has been characterized as a bactericide against *Erwinia* spp., *Xanthomonas* spp., and *Pseudomonas* spp. (Dimopoulou et al., 2021). This fast-growing soil bacterium establishes its advantage based on different modes of antagonistic action such as competition for mineral elements and space, excretion of antimicrobial metabolites, volatile organic compounds (VOCs) biofilms, siderophores and long shelf life based on spore formation ability (Dimopoulou et al., 2021; Ongena and Jacques, 2008).

The evaluation of the antifungal activity of *B. amyloliquefaciens* D5 ARV against *F. oxysporum*, *F. graminearum*, *B. cinerea*, and *Macrophomina* sp. was the main goal of the presented study. The modes of action were determined using confrontation tests and by observing the influence of non-volatile and volatile metabolites on phytopathogen growth and development.

2. Materials and methods

2.1. Confrontation test

The test was conducted with *Bacillus amyloliquefaciens* D5 ARV (Karličić et al., 2017) from the collection of the Department of Environmental Microbiology, Faculty of Agriculture (Belgrade, Serbia), and *Fusarium oxysporum*, *F. graminearum*, *Botrytis cinerea*, and *Macrophomina* sp. from the collection of the Institute for Plant Protection and Environment (Belgrade, Serbia). A nutrient medium used in the test was potato dextrose agar (PDA, Sigma-Aldrich, USA), which was inoculated with mycelium agar discs (5 mm diameter), while a line of *B. amyloliquefaciens* was streaked at a 3 cm distance. The control plates contained only fungi. Each treatment was carried out in triplicate at 25°C, until the plate's edge was reached by the control fungus.

The following formula was used to determine the percentage of mycelial growth inhibition (MGI):

$$\text{Mycelial growth inhibition (\%)} = ((\text{ØC} - \text{ØT})/\text{ØC})) \times 100$$

ØC – average diameter of a fungal colony of the control group,

ØT – average diameter of a fungal colony of the treatment group.

The degree of antagonistic activity was estimated according to Ruiz-Gómez et al. (2021) as: very high (% > 75), high (% = 61-75), moderate (% = 51–60), and low (% < 51).

2.2. The effects of B. amyloliquefaciens D5 ARV non-volatile metabolites on the pathogen's mycelial development

The non-volatile metabolites were prepared by growing *B. amyloliquefaciens* D5 ARV in nutrient broth (NB, Himedia, India) for 48 h. Afterward, centrifugation (Eppendorf, Germany) was used to remove the cells from suspension. One half of the obtained aliquot was sterilized through 0.2 µm filters (Sigma-Aldrich, USA) and the other half was autoclaved. The antifungal effects of metabolites were detected by adding 1, 5, and 10% of filter-sterilized or autoclaved non-volatile metabolites into PDA followed by inoculation with mycelia discs (5 mm diameter) of *F. oxysporum*, *F. graminearum, B. cinerea*, and *Macrophomina* sp. and incubation at 25°C. The control plates contained PDA. The experiment was set up in triplicate. Using the

previously given equation (section 2.1.), the impacts of non-volatile metabolites were approximated as a percentage of MGI.

2.3. The effect of B. amyloliquefaciens D5 ARV VOCs on the pathogen's mycelial development

The impact of bacterial VOCs on mycelial growth was examined by the confrontation test without contacts (Dennis and Webster, 1971). PDA plates were inoculated with pathogen's mycelia discs (5 mm diameter) and 20 µl of 24-old *B. amyloliquefaciens* D5 ARV culture. The inoculated plates were arranged to confront one another, sealed with Parafilm®, and incubated at 25°C until the plate's edge was reached by the control fungi. The experiment was set up in triplicate. Using the above given equation (section 2.1), the impacts of non-volatile metabolites were approximated as a percentage of MGI.

2.4. Collection of VOCs and GC-MS analysis

To determine VOCs emitted by *B. amyloliquefaciens* D5 ARV, penicillin bottles containing 5 ml MS medium (1.5% sucrose, 1.5% agar, 0.4% Tripton soy broth) were used. Six days afterward, the VOCs were collected by headspace solid-phase microextraction followed by gas chromatography (Agilent Technologies 7890 B GC System, AIM, Littleton, CO, USA) coupled with mass spectrometry (Agilent Technologies 5977A MSD, AIM, Littleton, CO, USA).

In summary, 0.2 g of the sample (MS + *B. amyloliquefaciens* D5 ARV) was added to a headspace vial together with 0.5 mL of sterile distilled water. Negative controls were bottles with sterile PDA. A cap with PTFE/silicone septa was used to seal each vial, followed by incubation at 70°C. The solid phase microextraction fiber (Polydimethylsiloxane (PDMS) 100 µm, Agilent Technologies, AIM, Littleton, CO, USA) was inserted into the headspace of the vial containing the sample solution. The extraction was carried out at 70°C with 90 min of fiber-exposed time. After sampling, the SPME fiber was withdrawn into the needle, removed from the tube, and inserted into the hot injector port (270°C) of the GC system where the extracted analyte was desorbed and transferred to the analytical column (HP-5, Agilent Technologies, AIM, Littleton, CO, USA). A relatively long desorption time in the injector (10 min) was selected to avoid carryover between runs to ensure full desorption of analyte from the fiber. Ultra-high purity 5.0 grade helium (Messer Tehnogas AD, Belgrade, Serbia) was used as a carrier gas run at a flow rate of 1.2 mL/min using splitless injection.

The oven was set to start at 50°C for two minutes, and then the temperature was raised in two steps: 50–80°C at a rate of 20°C/min and held for 6 min at this temperature; 80–280°C at a rate of 15°C/min and 240–280°C and held for 6 min at this temperature. Full scan mode (m/z 27-350) operated in the electron ionization mode at 70 eV with a source temperature of 230°C was used for data capture during the analysis. The National Institute of Standards and Technology (NIST) database was used to identify volatile compounds. The substances identified were defined as volatile organic compounds (VOCs) exhibiting mass spectra with a match factor of ≥80%.

3. Results and discussions

The biocontrol activity of *B. amyloliquefaciens* D5 ARV toward *F. oxysporum*, *F. graminearum*, *B. cinerea*, and *Macrophomina* sp. was estimated in the present study. Previous analyses grouped *B. amyloliquefaciens* D5 ARV in PGPB with the ability to express direct (production of ammonia, indole-3-acetic acid) and indirect mechanisms of plant growth promotion such as the production of siderophores, protease, and cellulase (Karličić et al., 2017; Karličić et al., 2020). The first step in the further determination of *B. amyloliquefaciens* D5 ARV antifungal mechanisms was the confrontation test against *F. oxysporum*, *F. graminearum, B. cinerea*, and *Macrophomina* sp. Table 1 displays the results that were attained after the incubation.

Table 1.
Mycelial growth inhibition (%) by *B. amyloliquefaciens* D5 ARV in a confrontation test

Pathogen	MGI (%)
Fusarium oxysporum	60±2.3
Fusarium graminearum	46±1.7
Botrytis cinerea	67±2.0
Macrophomina sp.	33±3.1

The confrontation test with *B. cinerea* reached the highest level of growth inhibition; according to the Ruiz-Gómez et al. (2021) classification, this level of antagonistic activity is estimated as high. The results obtained in a dual test with *B. cinerea* are in line with Ahlem et al. (2012), who reported nine isolates of *B. amyloliquefaciens* with antagonistic potential. Also, Haidar et al. (2016) marked *B. amyloliquefaciens*, together with *B. subtilis* and *B. megaterium*, as efficient BCAs against *B. cinerea*.

A low level of antagonistic activity was displayed by *B. amyloliquefaciens* D5 ARV versus *F. graminearum* and a moderate level toward *F. oxysporum*. This is in accordance with Uwaremwe et al. (2022), who reported the BCA potential of *B. amyloliquefaciens* HSB1 and FZB42 against several *Fusarium* species. *Bacillus* sp. is already well known by its ability to reduce the severity of *F. graminearum* infections (Abbas and li-Mattila, 2022). Jimenez-Quiros et al. (2022) reported the antagonistic effect of several *Bacillus* strains on *F. graminearum*. Those isolates were applied in the form of different dilution series and, compared to the results obtained in our study, showed significantly higher antagonistic potential, phytopathogen growth inhibition reached 79.30% (Jimenez-Quiros et al., 2022). Djordjević et al. (2011) emphasized the importance of natural enemies inhabiting the rhizosphere of diseased crops, but without underestimating the importance of antagonistic activity that can be expressed by non-soilborne bacteria such as the isolate used in this study.

In the confrontation test with *Macrophomina* sp., antagonistic activity was reported and estimated as low. The antagonistic activity of *Bacillus* sp. against *M. phaseolina* was confirmed by Rangel-Montoya et al. (2022). Torres et al. (2016) noted a 50% decrease in *M. phaseolina* growth induced by the presence of *B. amyloliquefaciens* PGPBacCA1, while Rangel-Montoya et al. (2022) reported 66.80% inhibition.

The results of the confrontation test showed different strategies approached by *B. amyloliquefaciens*

D5 ARV. The confronted cultures test with *Macrophomina* sp. showed the presence of an inhibition zone which suggested deadlock at distance mechanism (Krause et al., 2020) caused by the production of antifungal compounds, while *B. cinerea*, *F. oxysporum* and *F. graminearum* stopped their growth at contact with *B. amyloliquefaciens* D5 ARV.

The detection of mechanisms involved in BCA activity included the estimation of the antifungal potential of *B. amyloliquefaciens* D5 ARV non-volatile and volatile metabolites (Table 2). Non-volatile metabolites were subjected to two different approaches of sterilization, by filtration, and autoclaving. The findings indicated no significant effects of autoclaved metabolites in the sense of mycelial growth inhibition. The visible changes occurred in the case of *F. graminearum* and *Macrophomina* sp., where mycelium density was decreased. On the other hand, filter-sterilized metabolites expressed some level of inhibition, which increased with the increase in the concentration of filter-sterilized metabolites. Such effects confirmed the presence of antifungal compounds in their composition. A comparison of filter-sterilized and autoclaved metabolites suggests that antifungal compounds are heat sensitive and that autoclaving causes the inactivation of metabolites with antifungal activity. This is in accordance with Helbig and Bochow (2001), who reported that the antifungal activity of *B. subtilis* culture filtrate against *B. cinerea* was lost after autoclaving.

All concentrations of *B. amyloliquefaciens* D5 ARV filter-sterilized metabolites showed a low level of antagonistic activity toward *B. cinerea* according to Ruiz-Gómez et al. (2021). The highest percentage of inhibition was achieved at the concentration of 10%, as it was noted with the other tested pathogens (Table 2). On the other hand, Chen et al. (2019) demonstrated significant antifungal activities of *B. amyloliquefaciens* RS-25 culture filtrates against *B. cinerea*.

Table 2.
Mycelial growth inhibition (%) by *B. amyloliquefaciens* D5 ARV non-volatile and volatile (VOC) metabolites

Pathogen		Mycelial growth inhibition (%)			
Concentration of metabolites (%)		*Botrytis cinerea*	*Fusarium oxysporum*	*Fusarium graminearum*	*Macrophomina sp.*
Filter-sterilized metabolites	1	12±1	29±1	25±2	Less mycelium
	5	35±2	47±2	37±3	35±3
	10	45±2	53±2	50±2	78±2
Autoclaved metabolites	1	No inhibition	No inhibition	Less mycelium	Less mycelium
	5	No inhibition	No inhibition	Less mycelium	Less mycelium
	10	No inhibition	No inhibition	Less mycelium, no pigment	Less mycelium
VOC		53	30	47	/

The presence of filter-sterilized metabolites at a concentration of 10% showed a moderate level of antagonistic activity toward *F. oxysporum* and *F. graminearum*, and that is the same effect as in the confrontation test, suggesting that compounds in the filtrate are, potentially, the main carriers of antifungal mechanism in those cases. The results of Jimenez-Quiros et al. (2022) are in line with ours, and they reported the antagonistic activity of cell-free filtrates of several *Bacillus* strains against *F. graminearum* with the percentage of growth inhibition ranging from 58.33 to 70.07%. In our study, 10% of the filtrate was enough to cause growth inhibition of *F. oxysporum* higher than 50%, while Jangir et al. (2021) recorded such an effect in the case of *B. subtilis* using a much higher concentration of culture filtrates, 40%. This emphasizes the importance of detecting and separating active compounds, bearing in mind that they are diluted in such mixtures.

The most interesting result was recorded in the case of *Macrophomina* sp., where the presence of 10% filter-sterilized metabolites caused very high antagonistic activity (Figure 1).

The inhibition zone noted in the confrontation test indicated the presence of toxic compounds affecting *Macrophomina* sp. Those results suggest that secondary metabolites are, probably, the main mechanisms of antagonistic activity exhibited within this interaction. Rangel-Montoya et al. (2022) also recorded the inhibition of mycelial growth, but also the inhibition of microsclerotia production and morphological changes of *M. phaseolina* induced by cell-free supernatants of two *B. amyloliquefaciens* strains. *Macrophomina* sp. grown on PDA supplemented with non-volatile metabolites gained brown color in the center of white colony and such observation was also noted by Rangel-Montoya et al. (2022).

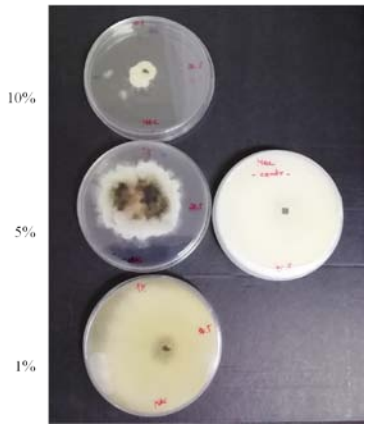

Figure 1. Effects of *Bacillus amyloliquefaciens* D5 ARV non-volatile metabolites on *Macrophomina* sp. growth

PGPB's VOCs expose beneficial effects by promoting plant growth, exhibiting antifungal activity, and inducing systemic resistance (Tahir et al., 2017). The volatile metabolites disperse widely and form a fungistatic environment. Unlike them, non-volatile metabolites act locally. It is also confirmed that

bacterial VOCs exert a lethal effect on spore survival in the soil and limit the occurrence of the disease (Yuan et al., 2012). The role of volatile substances in the overall antagonistic effect expressed by *B. amyloliquefaciens* D5 ARV was also examined in this work. Figure 2 displays the released VOCs, and Table 3 provides a summary of them.

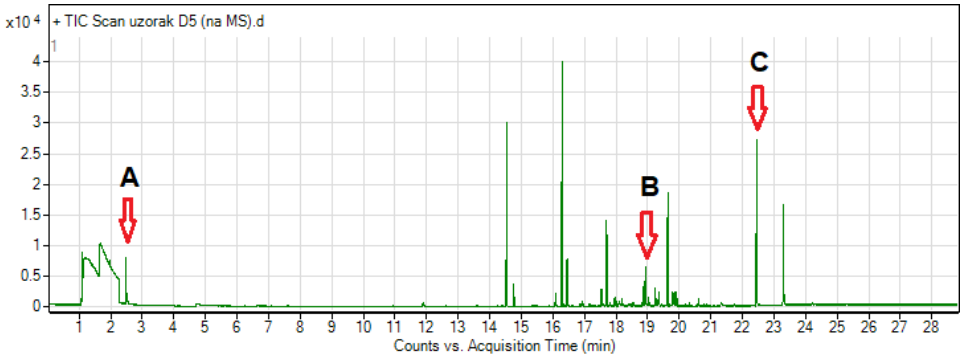

Figure 2. Representation of VOCs chromatographic peaks realized by *Bacillus amyloliquefaciens* D5 ARV in MS medium

In the case of *B. cinerea*, the presence of VOCs showed higher inhibitory potential compared to non-volatile metabolites, and the reported level of antagonistic activity was marked as moderate (Table 2). Chen et al. (2019) also confirmed that VOCs produced by the *B. amyloliquefaciens* and several other *Bacillus* isolates inhibit *B. cinerea* growth. In the case of *F. oxysporum* and *F. graminearum*, the produced VOCs demonstrated antifungal activity but the level was lower compared to the previous two tests. Similar results were obtained by Wu et al. (2019), who confirmed that both non-VOCs and VOCs of *B. amyloliquefaciens* L3 expressed antifungal effects, particularly against *Fusarium oxysporum* f. sp. *niveum.* Yuan et al. (2012) reported 32% reduction in *F. oxysporum* mycelial growth by the *B. amyloliquefaciens* NJN-6 VOCs. Additionally, the same authors confirmed that VOCs inhibited the germination of *F. oxysporum* spores. No inhibition of the mycelial growth of *Macrophomina* sp. was reported after exposure to VOCs. On the contrary, Rangel-Montoya et al. (2022) reported that the VOCs of two strains of *B. amyloliquefaciens* inhibited mycelial growth and the production of microsclerotia of *M. phaseolina*.

Table 3. The VOCs emitted by *Bacillus amyloliquefaciens* D5 ARV

Sample	Retention time (min)	Peak	Volatile compound	Relative abundance
B.amyloliquefaciens D5 ARV	2.491	A	Silanediol, dimethyl	3.59
	19.029	B	Pentadecanoic acid	2.53
	22.447	C	Hexanedioic acid, bis(2-ethylhexyl) ester	12.83

The VOCs identified in this study are well-known as part of the *Bacillus* sp. VOCs spectra (Tahir et al., 2017, Akpor et al., 2021, Mohamad et al., 2018). Silanediol, dimethyl is known for its potency against the activity of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (Femi-Adepoju et al., 2018). The VOCs of *B. amyloliquefaciens* FZB42 decreased the virulence and motility of *Ralstonia solanacearum*, and silanediol, dimethyl was among the detected compounds (Tahir et al., 2017). Pentadecanoic acid and hexanedioic acid, bis(2-ethylhexyl) ester are well-known as antifungal compounds (Nakkeeran et al., 2020), and the antifungal activity of hexanedioic acid, bis(2-ethylhexyl) ester against *Fusarium* sp. has been documented by Elleuch et al. (2010). Also, those two compounds were detected in a plant extract that showed high effectiveness against *Slerotium rolfsi* (Derbalah et al., 2012).

Volatile metabolites of *Bacillus* sp. are well-known and described; in the case of *B. amyloliquefaciens,* several VOCs, such as acetoin and 2,3-butanediol, have been confirmed to be plant growth promoters (Wu et al., 2019). Rangel-Montoya et al. (2022) confirmed that the VOCs produced by several *B. amyloliquefaciens* strains inhibited the growth of *F. oxysporum*, *Rhizoctonia solanacearum*, *B. cinerea*, *Sclerotinia sclerotiorum*, *Verticillum longisporum*, and *M. phaseolina*.

4. Conclusions

In this study, the previously described PGPB *B. amyloliquefaciens* D5 ARV was further examined in terms of antifungal potential toward *B. cinerea*, *F. oxysporum*, *F. graminearum,* and *Macrophomina* sp. The confrontation test revealed high antagonistic activity against *B. cinerea*, while very high antagonistic activity was performed by *B. amyloliquefaciens* D5 ARV non-volatile metabolites toward *Macrophomina* sp., suggesting that metabolites are the main carriers of antifungal mechanism. The compounds detected in the VOCs spectra were already documented as antimicrobial substances. The obtained results suggested that effects were obtained by the combined action of non-volatile and volatile compounds, among other potential mechanisms. On the other hand, undoubtedly, *B. amyloliquefaciens* D5 ARV VOCs had no effects on *Macrophomina* sp. mycelial growth.

The results of this study confirmed *B. amyloliquefaciens* D5 ARV to be a BCA underlining the potential visible through the interactions with *B. cinerea* and *Macrophomina* sp. Future research should focus on *in planta* research and detection of particular metabolites that are the main carriers of biocontrol activity.

Acknowledgment

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (contract No. 451-03-65/2024-03/200116).

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