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Cell-free filtrates from plant pathogens: Potential new sources of bioactive molecules to improve plant health

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ABSTRACT

Lack of alternatives to reduce the use of and risk by agrochemicals makes necessary to search for environmentally friendly and health-safe options to increase crop production. The use of beneficial microorganisms in agriculture offers a sustainable alternative to the use of chemicals. However, only a few microbe-based commercial products are available on the market due to limitations associated with the microbial growth in artificial media, survival, and performance in different environmental conditions. Use of microbial cell-free broth cultures (known as cell-free filtrates: CFFs) from plant pathogens offers several advantages over, and reduces the limitations of, traditional microbe-based products. A large diversity of secondary metabolites and bioactive molecules are secreted by plant pathogens and such metabolites represent a large reservoir of compounds with potential for use in crop growth and crop protection. The objective of this review is to provide an updated compilation and discussion of the published literature on CFFs from phytopathogenic microorganisms. Different growth conditions of microorganisms and ways of applying their CFFs in the studies are shown, since the accumulation of bioactive compounds in CFFs depends on factors such as the composition of the culture medium or the culture temperature. Mechanisms and molecules related to CFFs bioactivity are discussed, evidencing the complexity of the filtrate-plant interaction network. This review underlines the potential of CFFs as an alternative source to plant health in the sustainable crop production system of the future, and it opens the door for their application in other unexplored fields.

1. Introduction

Today's agriculture faces significant challenges that threaten the global food supply (De Clercq et al., 2018). By 2050, the world population will reach 9.2 billion people, increasing food demand by 59–102%. This change implies the need to improve agricultural productivity by 60–70%, which relies principally on increasing crop yields, as the arable land area cannot be further increased (Pawlak and Kołodziejczak, 2020). Such process will require a sustainable approach that increases productivity and, whenever possible, generates social and environment benefits (Rose and Chilvers, 2018).

In addition, agricultural productivity nowadays is being reduced because of climate change through extreme abiotic stresses caused by elevated temperatures, salinity/alkalinity, drought/waterlogging, and abrupt rainfall patterns (Shahzad et al., 2021). Furthermore, the rise in global temperature will lead to an increase in the geographic distribution of agricultural pests and pathogens (Skendžić et al., 2021) and biotic stresses that today account for losses of global crop yield of 10–25% (Poveda, 2021a; Mohammad-Razdari et al., 2022). To combat them, the strategy most frequently used is the application of chemical pesticides. However, the pesticide wide use can cause serious environmental and health problems (Tudi et al., 2021). Lack of alternatives to reduce the reliance on pesticides in agriculture makes necessary the search for environmentally friendly and health-safe options to increase crop

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production in a sustainable way (Jhariya et al., 2021).

One of the strategies to cope with some of the current problematic in agriculture includes the use of beneficial microorganisms that contribute to maintaining soil fertility and crop health in an eco-friendly way (Yadav, 2021). Hence, microorganisms of interest can be identified and used as agricultural bioinoculants (Singh et al., 2021). Among them are those known as plant growth promoting microorganisms (PGPMs) or biofertilizers, characterized by the direct supply and/or mobilization of nutrients to plants and by enhancing the production of plant growth hormones, such as auxins, cytokinins, abscisic acid, gibberellins and ethylene (Martínez-Viveros et al., 2010; Sharma and Kaur, 2017; Khan et al., 2019; Prasad et al., 2019; Hakim et al., 2021). The PGPMs can improve crop quality by increasing the nutrient and nutraceutical metabolite content of foods (Ganugi et al., 2021). Other microorganisms may also be able to increase plant tolerance under abiotic stresses, such as drought, salinity, or heat (Hakim et al., 2021). Biofertilizing microorganisms and those that improve crop quality and tolerance to abiotic stresses are called microbial biostimulants according to European Union legislation (Poveda and González-Andrés, 2021). On the other hand, there are both beneficial and pathogenic microorganisms that produce secondary metabolites and volatile compounds that have potential for use as plant growth promoters and biological control agents (Pirttilä et al., 2021; Poveda, 2021b; Gámez-Arcas et al., 2022). However, although that type of microorganisms is a promising alternative to chemical fertilizers and pesticides, they have some limitations, due to the inoculation efficiency and the complexity of cultivation of many strains at an industrial level (Pellegrini et al., 2020; dos Santos Lopes et al., 2021). Additional limitations include their isolation, identification and growth at an adequate concentration, the antagonistic effects of some molecules, or insufficient knowledge about how plants perceive and react against some of microbe-derived compounds (Naamala and Smith, 2021).

An alternative to the drawbacks mentioned above is the culture of microorganisms in liquid medium and their subsequent filtration to remove all living cells (Fig. 1). Then, a liquid fraction rich in bioactive chemicals is obtained. This has been referred to as filtrates, cell-free cultures/filtrates/supernatants, exudates, non-volatile metabolites/compounds, soluble metabolites/compounds or diffusible metabolites/compounds. The majority of studies describe the positive effect of the application of microbial cell free filtrates (CFFs) from beneficial plant microorganisms on biocontrol treatment for plant pathogens (Mathivanan et al., 2008; Wu et al., 2015; Meena et al., 2017; Pellegrini et al., 2020; Wang et al., 2022). Moreover, these CFFs promote growth and enhance the yield of crops (Aldesuquy et al., 1998; Varma et al., 1999; Bagde et al., 2011; Sung et al., 2011; Rahman et al., 2012; Kaur et al., 2019; Pellegrini et al., 2020; Yandigeri et al., 2012). Thus, CFFs from beneficial microbes can be an environmentally friendly approach to increase crop protection and crop productivity. However, recently published studies show evidence that the positive effect of CFFs also extends to phytopathogenic microorganisms.

Thus, the aim of this review was to compile and analyze most, if not all, so far published studies on CFFs from phytopathogenic microorganisms and their potential application in crop health. The review discusses the role of extracted filtrates molecules of crop production interest and their potential contribution to the development of more sustainable and environmentally friendly methods on crop health.

2. CFFs from plant pathogens

For decades, CFFs from plant-pathogens have been used to characterize and describe possible molecules involved in plant infection (Dow and Callow, 1979; Huet et al., 1992; Inbar and Chet, 1994; Wilson et al., 2002; Tsuge et al., 2013). However, their possible beneficial role on crops has not been explored, and only a few studies have addressed their effect on plant growth and development. In the CFFs production process, it is important to remark the necessity of the last filtration step (Fig. 1) when working with a plant phytopathogen to avoid releasing the microorganism in the environment.

In the following sections, we will discuss the results of different studies on CFFs as phytotoxic compounds and their possible benefits to crop growth and its tolerance/resistance to abiotic and biotic stresses. The section also summarizes the effects and mechanisms of such types of compounds (Fig. 2).

2.1. *Phytotoxicity of CFFs from plant pathogens*

Liquid culturing of plant-pathogenic microorganisms and subsequent culture filtration is the most widely used methodology to obtain and characterize pathogen phytotoxins (Strange, 2007). Culture filtrates have been widely described as capable of causing disease in plant tissues by themselves (Erikson and Montgomery, 1945; Bonnet and Rousse, 1985; Tomas and Bockus, 1987; Bailey, 1995; Faris et al., 1996). This is due to the pathogen production and release of different secondary metabolites and primary molecules that act as phytotoxins (Strange, 2007).

Examples of compounds found in CFFs include thaxtomin A, produced by the bacteria *Streptomyces acidiscabies,* causal agent of scab disease in potatoes (Healy et al., 2000), citrinin, fusaric acid and radicicol, caused by the soilborne fungus *Fusarium virguliforme*, causal agent of sudden death syndrome of soybean (Chang et al., 2016), and the phytotoxic protein PcF, produced by the oomycete *Phytophthora cactorum,* associated to necrosis in strawberry leaves (Orsomando et al., 2001).

Due to their phytotoxic effect, CFFs of different plant-pathogens have been long used for selection of resistant plant cell lines in generating resistant breeding lines or whole plants with true agricultural use (Švábová and Lebeda, 2005). In a study with proembryogenic masses of grapevine 'Chardonnay' cells, *Elsinoe ampelina* resistant lines were obtained by growing the plant cells in a medium containing 40% fungal

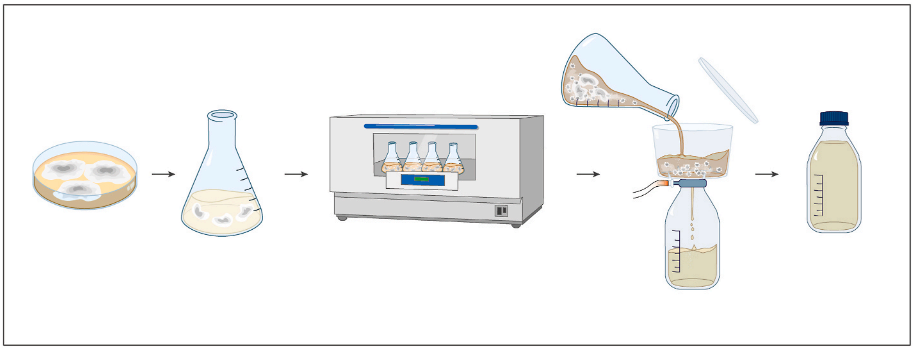

Fig. 1. Laboratory scale production process of CFFs. The process starts with the microorganism solid culture prior to its cultivation in liquid media. This liquid culture is incubated under optimum parameters as long as is required and finally submitted to filtration, obtaining a sterile fraction free of living cells.

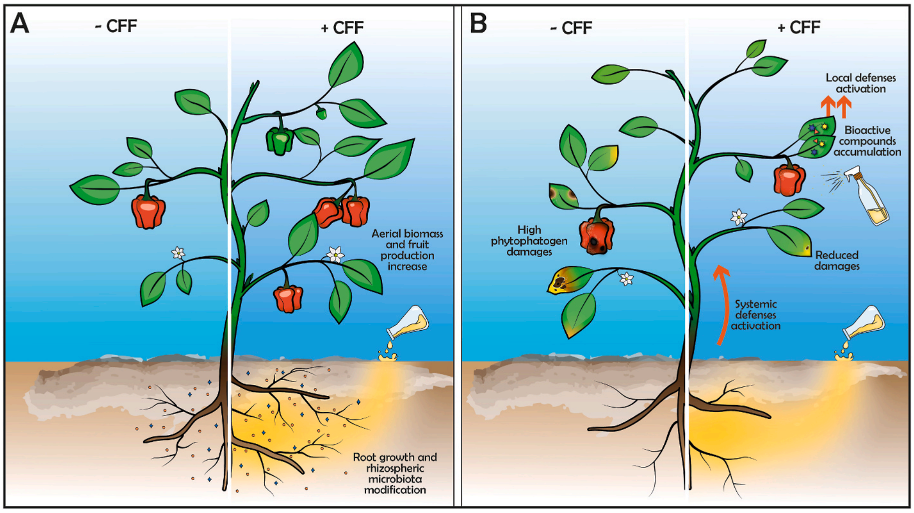

Fig. 2. Infographic of how the application of CFFs benefits crop yield under control and against biotic stresses conditions. (**A**) shows how the soil application of CFFs leads to metabolic changes that increase crop yield (aerial and root biomass and fruit production) and soil rhizosphere beneficial microorganisms. (**B**) shows how soil and foliar application of CFFs reduce the phytopathogen damage in crops due to the activation of systemic and/or local defense responses (accumulation of bioactive compounds).

culture filtrates. These new resistant lines were the consequence of a constitutive and heritable increase in plant chitinase activity (Jayasankar et al., 2000). However, the biological material more commonly used in the selection of resistant lines by exposure to CFFs from plant pathogens is a callus. For example, the application of CFFs from *Fusarium oxysporum* in cotton calli, and the contact with CFFs from *Alternaria carthami* in safflower, leaded to an increase in chitinase activity and superoxide dismutase activity, respectively (Ganesan and Jayabalan, 2006; Vijaya-Kumar et al., 2008). In addition, seeds have been used directly in the selection of resistant lines using this methodology. The application of CFFs from the soilborne pathogen *Pyrenochaeta lycopersici* on tomato seeds allowed the selection of resistant lines rapidly, with disease symptoms appearing in the first rootlets of susceptible lines (Fiume and Fiume, 2003).

2.2. *CFFs from plant pathogens as biostimulants of plant growth*

Following the methodology described in the previous section, nucellar calli from orange and lemon trees were exposed to CFFs from the citrus pathogen *Phoma tracheiphila*. Surprisingly, lemon calli treated with 50% CFF showed a significant increase in biomass compared to the untreated control. This effect was due to the pathogen production and release of IAA in the culture medium (Gentile et al., 1992). This was the first description of a plant growth promoting effect of CFF from a plant pathogen.

A study with the model plant *Arabidopsis thaliana* found that application of CFFs from different plant-pathogens promoted *Arabidopsis* plant growth (Ávila and Poveda, 2021). Specifically, root application of CFFs from the bacteria *Pectobaterium carotovorum* and *Pseudomonas syringae* pv. tomato, the fungi *F. oxysporum* f.sp. *conglutinans* and *Rhizoctonia solani*, and the oomycete *Phytium irregulare*, caused a significant increase in plant aerial and root biomass. However, the treatment did not result significant differences in fruit formation compared to untreated plants. Furthermore, in that study, it was observed that CFFs from the pathogenic fungus *Sclerotinia sclerotiorum* did not have a plant growth promoting effect (Ávila and Poveda, 2021).

A recent study by Baroja-Fernandez et al. (2021) showed that soil application of CFFs from the pathogenic fungi *Alternaria alternata* and *Penicillium aurantiogriseum* significantly increased root biomass and fruit production of pepper plants (represented in Fig. 2A). They also improved fruit sugar and amino acid content. Furthermore, distillation of these CFFs showed that the VCs present in the pathogen filtrates also increased the growth and productivity of bell pepper plants. Finally, it was described how both the CFFs of both pathogens and their VCs modify the rhizospheric microbiota, increasing its content in plant-beneficial bacterial and fungal taxa (Baroja-Fernandez et al., 2021). These results demonstrate that the CFFs of phytopathogenic microorganisms can have a positive effect on the plant metabolism and development. However, the production of bioactive compounds depends on factors such as the composition of the medium and the experimental conditions used (Ogórek, 2016; Baroja-Fernandez et al., 2021; Morcillo et al., 2022). For example, CFFs from the phytopathogen fungus *A. alternata* cultures in Richard's solution had a negative effect on the seed germination of different crops (Parveen et al., 2019). On the contrary, CFFs from the same fungus cultured in MS medium had a positive effect on the growth and yield of the crop (Baroja-Fernandez et al., 2021). Moreover, Baroja-Fernandez et al. (2021) reported that the effect of CFFs was positive or negative depending on the age of the microbial culture. Therefore, phytotoxin formation by microbes depends on diverse environmental factors, including the microbial culture medium composition, culturing duration and other conditions (temperature, light, etc.). The data obtained by Baroja-Fernandez et al. (2021) show that the microorganisms, cultured under specific conditions, are not active in phytotoxin production and exert a positive effect in the crops. Moreover, Javaid et al. (2017) showed that CFFs from *Alternaria japonica* had a higher phytotoxic activity when cultured in potato dextrose broth medium compared to the ones grown in malt extract broth.

2.3. *CFFs from plant pathogens improve plant health*

Plant health may be compromised by different abiotic or biotic stresses. Little information exists on the potential of CFFs from plant pathogens in enhancing plant tolerance to abiotic stresses. Poveda (2022) carried out a study in tomato calli and CFFs from the cruciferous pathogenic fungus *Leptosphaeria maculans*. Under both drought and salinity conditions, the CFFs increased the growth and vitality of tomato calli and reduced the oxidation and production of reactive oxygen species (ROS). In addition, the application of these CFFs in tomato calli reduced the expression of genes related to plant stress and increased the expression of genes related to tolerance under abiotic stresses, such as *AREB1* (Poveda, 2022). The most studied application for CFFs from plant-pathogens so far is as a biological control strategy, both directly, through their antimicrobial and phytotoxic activity, and indirectly, through the activation of plant defenses (Fig. 2B). Important results have been obtained with plant pathogen CFFs in order to mitigate biotic stress. Table 1 shows the compilation of these works.

Recently, CFFs from various plant-pathogenic fungi forming sooty molds on infected plant organs have been tested. Specifically, CFFs were obtained from the plant pathogens *Trichomerium deniqulatum*, *Capnodium* sp. and *Leptoxyphium* sp., extracting the metabolites present with ethyl acetate. The different extracts obtained were applied on different plant pathogens *in vitro*. It was shown that the total phenolic content and 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) present in the CFFs were responsible for inhibiting the growth of the pathogens *Alternaria* sp. and *Curvularia* sp. However, the flavonoid content of CFFs was associated with growth promotion of the pathogens *Fusarium* sp., *Colletotrichum* sp. and *Pestalotiopsis* sp. (Haituk et al., 2022). Similar procedure was followed by Pacios-Michelena et al. (2023), confirming the presence of antifungal compounds in CFFs from *Penicillium chrysogenum*, including 1,4-benzoquinone imine, viridicatic acid, phenol-5-methyl-2-(1-methyl ethyl), and hydrolytic enzymes β 1–3 glucanase and chitinase. The antifungal activity reduced *in vitro* growth of other phytopathogens of economic importance in agriculture, such as *R. solani*, *Phytophthora* sp., *Botrytis cinerea* and *F. oxysporum*. Not only *in vitro* assays, but also pathogen-inoculated fruits tests have been done (Hassine et al., 2022). In this case, CFFs from *Penicillium* sp. fungi reduced anthracnose disease (*Colleotrichum cocodes*) severity in tomato fruits compared to the control.

Therefore, CFFs from plant-pathogens can be used for biological control strategies against certain pathogens, but also may favor the growth of others. Thus, further research on the metabolites obtained and their application in field conditions is required.

Like the interaction among microorganisms, plants and microorganisms have also evolved a complex communication system based on different chemical signals. This interkingdom communication can benefit both sender and receiver or only one of them, developing coercive interactions. This would be the case of the release of phytohormones by microorganisms or the manipulation of microbial quorum-sensing compounds by plants (Rowe et al., 2018).

The activation of plant defenses through different elicitors produced by phytopathogen microorganisms has been considered for a long time. By 1992, some studies had already tested the phytotoxic effect of different elicitins from the fungus-like oomycete *Phytophthora* sp. (Huet et al., 1992). Apart from the necrotic properties of these holoproteins, they are also known for the activation of plant defense responses, leading to a protection against pathogens infections.

In a plant-pathogen interaction, there is a continuous evolutionary struggle of attack/defense by both protagonists. Plant cells have pattern recognition receptors (PRRs) in their cell membranes that recognize molecular components released or present in plant-pathogen microorganisms, known as pathogen-associated molecular patterns (PAMPs) (Amari and Niehl, 2020; Poveda, 2020). In response, the plant activates its defenses through the PAMP-triggered immunity (PTI). On the other hand, pathogens can interfere with immune signaling and even block it through different effector proteins, a mechanism called effector-triggered susceptibility (ETS) (Poveda, 2020; Nguyen et al., 2021). However, the plant can recognize pathogen effectors and prevent their action, which leads to the activation of a new specific defense response called effector-triggered immunity (ETI). All these plant defensive responses are mediated by different hormonal pathways that are distributed throughout the plant, developing a systemic resistance against the pathogen (Poveda, 2020; Remick et al., 2023). In this sense, CFFs produced by pathogens can contain a wide variety of these PAMPs and be used as "plant vaccines". Indeed, boiled CFFs have activated local plant defense responses, as with the pathogenic bacterium *Ralstonia solanacearum* in tobacco and *A. thaliana* plants (Pfund et al., 2004).

Some of these PAMPs related to the activation of local defenses by CFFs from plant-pathogens have been characterized. In bean plants, CFFs from *Colletotrichum lindemuthianum* induce a local accumulation of phytoalexins in cotyledons and hypocotyls due to the presence of a polysaccharide rich in glucan (Anderson-Prouty and Albersheim, 1975). This defensive response has also been observed in soybean plants treated with CFFs from *P. carotovorum*, but as a consequence of the action of the bacterial endopolygalacturonic acid lyase enzyme, which releases plant cell wall fragments (Davis et al., 1984).

Plants exposed to CFFs respond by increasing the local expression of different defense-related genes (Ponce de León et al., 2007; McLellan et al., 2013). For example, CFFs from the oomycete *Phytophthora infestans* increase the expression of *NTP1* and *NTP2* genes in *Nicotiana benthamiana* leaves upon infiltration (McLellan et al., 2013). Sprayed CFFs from *P. carotovorum* increase the local expression of *PAL*, *CHS* and *LOX* genes in moss *Physcomitrella patens*, reducing infection by the pathogenic bacterium and by the fungus *B. cinerea* (Ponce de León et al., 2007).

In addition to a local activation of plant defenses, these CFFs can induce the activation of systemic resistance. CFFs from *Colletotrichum acutatum* were sprayed on strawberry plants prior to infection with the pathogen *B. cinerea*, which resulted in a reduction of the pathogen damage through local and systemic activation of plant defenses related to the expression of *ETR1*, *ERS1*, *ERF1* and *GLS5* genes (Tomas-Grau et al., 2020). *P. carotovorum* CFFs induce local and systemic accumulation of 3-indolylmethylglucosinolate and the phytoalexin camalexin upon droplets on *A. thaliana* leaves. Both the local and systemic defense response of secondary metabolite accumulation was JA-mediated (Brader et al., 2001). Specifically, it was described how different plant cell wall microbial lytic enzymes (pectinases and cellulases) accumulated in these CFFs, which were responsible for the activation of local and systemic defenses in the plant by releasing plant cell wall oligomers. This plant defensive response was quantified as an increase in gene expression of the defense enzyme β-1,3-glucanase locally and systemically, non-SA-mediated (Vidal et al., 1998). CFFs from *A. alternata* have been proven to reduce disease severity by inducing systemic defense response in *Catharanthus roseus* (Paul et al., 2022). In this case, the response was mediated by signaling molecule nitric oxide, along with higher activity of defense-related enzymes and the accumulation of total phenol and flavonoid content.

With respect to the activation of systemic resistance by plant-pathogens CFFs, only two studies have been conducted using root application and foliar response analysis. In *A. thaliana*, CFFs from various plant-pathogens (*P. carotovorum*, *P. syringae* pv. tomato, *F. oxysporum* f.sp. *conglutinans*, *R. solani*, *S. sclerotiorum* and *P. irregulare*) were used to activate systemic defenses against *B. cinerea*. All CFFs were reported to increase significantly the expression of SA (*PR-1*) and JA (*VSP2*) response genes when infected with the pathogen, compared to plants without CFFs application, leading to a lower infective capacity of *B. cinerea* (Ávila and Poveda, 2021). With CFFs from *Sclerotinia rolfssi* applied to chickpea plants, it was found that the activation of systemic defensive responses was SA-mediated and led to an accumulation of phenolic compounds in the aerial tissues (Singh et al., 2003).

Due to the possible phytotoxic activity of CFFs from plant pathogens mentioned in section 2.1, they could also be used to reduce biotic stress caused by weeds in crops. Weeds can produce the highest potential loss (34%) among crops, affecting food production in agricultural systems, decreasing the product quality and productivity due to the competition for natural resources (Oerke, 2006; Monteiro and Santos, 2022). Thus, sustainable strategies to control weeds in a more environmentally friendly way may be needed.

One example of these weeds is parthenium (*Parthenium hysterophorus*), a devastating weed of many economically important crops responsible for significant loses in the agricultural sector. Kausar et al. (2022) evaluated the herbicidal potential of the CFFs from *Alternaria brassicicola* and *A. gaisen* over this weed. The results showed that culture filtrates from both phytopathogenic fungi, especially *A. gaisen*, had significant herbicidal activity against *P. hysterophorus*, suppressing seed germination, root and shot growth of this weed. This effect was

Table 1
Use of CFFs from plant-pathogen microorganisms against plant biotic stresses.

PLANT-PATHOGEN		CULTIVATION CONDITIONS			PLANT	EXPERIMENT	CFF APPLICATION	BIOTIC STRESS	EFFECT	REFERENCE
Bacteria	*Pectobacterium carotovorum* (before: *Erwinia carotovora*)	TSB medium	72h	100 rpm, 24 °C	Soybean	ND	Cotyledons cut surface application	–	Plant defense responses activation	Davis et al. (1984)
		LB medium	ND	28 °C	Tobacco	Growth chamber	Leaves infusing	–	Plant local and systemic resistance activation	Vidal et al. (1998)
		LB medium	ND	28 °C	*Arabidopsis thaliana*	Growth chamber	Droplets on leaves (2 μl)	*E. carotovora* (bacteria)	Plant local and systemic resistance activation	Brader et al. (2001)
		LB medium	ND	28 °C	*Physcomitrella patens* (moss)	Growth chamber	Plant spraying (187.5 μl per moss colony)	*E. carotovora* (bacteria) *B. cinerea* (fungus)	Plant defense responses activation	Ponce de León et al. (2007)
		LB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly (400 μl per plant)	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)
	Pseudomonas syringae pv. tomato	LB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly (400 μl per plant)	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)
	Ralstonia solanacearum	CPG broth	ND	28 °C	Tobacco *A. thaliana*	*In vitro*	Leaves infusing	*R. solanacearum* (bacteria)	Plant defense responses activation	Pfund et al. (2004)
Fungi	*Alternaria alternata*	PDB medium	30d	ND	*Catharanthus roseus*	*In vitro*	Over leave surface (15 μl)	*A. alternata (fungus)*	Plant defense responses activation	Paul et al. (2022)
	Alternaria brassicicola, Alternaria gaisen	MEB medium	15d	25 °C	-	*In vitro*	Impregnated onto sterile paper (2 mL)	*Parthenium hysterophorus* (weed)	Phytotoxic activity (reduce germination, root and shot growth)	Kausar et al. (2022)
	Alternaria japonica	Richard's broth	21d	30 °C	Several weeds	Growth chamber	Foliar spray	Several weeds	Phytotoxic symptoms	Dutta et al. (2015)
	A. japonica	MEB and PDB medium	14d	25 °C	*P. hysterophorus*	*In vitro* and growth chamber	Impregnated onto sterile paper (2,5 mL) and foliar spray	*P. hysterophorus* (weed)	Phytotoxic activity (reduce germination and seedling growth)	Javaid et al. (2017)
	Capnodium sp.	PDB medium	14d	120 rpm, 25 °C	–	*In vitro*	Impregnated onto sterile paper discs	*Alternaria* sp. (fungus) *Curvularia* sp. (fungus)	Direct antifungal activity	Haituk et al. (2022)
	Colletotrichum acutatum	PDB medium	10d	ND	Strawberry	Growth chamber	Plant spraying (run-off)	*B. cinerea* (fungus)	Plant local and systemic resistance activation	Tomas-Grau et al. (2020)
	Colletotrichum lindemuthianum	Medium complex with casein hydrolysate extract	8d	100 rpm, 23 °C	Bean	ND	Cotyledons and hypocotyls cut surface application	–	Plant defense responses activation	Anderson-Prouty and Albersheim (1975)
	Fusarium oxysporum f. sp. *conglutinans*	PDB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly (400 μl per plant)	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)
	Gliocladium spp., *Penicillium* sp.	PDB medium	15d	150 rpm, RT	-	*In vitro*	Mixed with PDA/ injected in tomato wounds (100 μl)	*Colletotrichum coccodes* (fungus)	Direct antifungal activity and reduced disease severity	Hassine et al. (2022)
	Leptoxyphium sp.	PDB medium	14d	120 rpm, 25 °C	–	*In vitro*	Impregnated onto sterile paper discs	*Alternaria* sp. (fungus)	Direct antifungal activity	Haituk et al. (2022)
	Penicillium chrysogenum R1	PDB medium	7d	130 rpm, 28 °C	-	*In vitro*	Mixed with PDA	*F. oxysporum* (fungus), *B. cinerea* (fungus), *Phytophtora* sp. (oomycete), *Rhizoctonia solani* (fungus)	Direct antifungal activity	Pacios-Michelena et al. (2023)

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Table 1 (*continued*)

PLANT-PATHOGEN		CULTIVATION CONDITIONS			PLANT	EXPERIMENT	CFF APPLICATION	BIOTIC STRESS	EFFECT	REFERENCE
	Rhizoctonia solani	PDB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly (400 µl per plant)	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)
	Sclerotinia sclerotiorum	PDB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly (400 µl per plant)	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)
	Sclerotium rolfsii	PDB medium	14d	25 °C	Chickpea	Greenhouse	Radicularly (10mµl per plant)	–	Plant systemic resistance activation	Singh et al. (2003)
	Trichomerium deniqulatum	PDB medium	14d	120 rpm, 25 °C	–	*In vitro*	Impregnated onto sterile paper discs	*Alternaria* sp. (fungus)	Direct antifungal activity	Haituk et al. (2022)
Oomycetes	*Phytophthora infestans*	PB medium	ND	ND	*Nicotiana benthamiana*	Greenhouse	Leaves infiltration	*P. infestans* (oomycete)	Plant defense responses activation	McLellan et al. (2013)
	Phytophthora megasperma var. *sojae*	Asparagine medium	14-21d	24 °C	Soybean	ND	Cotyledons cut surface application	–	Plant defense responses activation	Frank and Paxton (1971) Ayers et al. (1976)
	Phytium irregulare	PDB medium	48h	180 rpm, 28 °C	*A. thaliana*	Growth chamber	Radicularly	*B. cinerea* (fungus)	Plant systemic resistance activation	Ávila and Poveda (2021)

CDB: Czapek Dox broth.
CPG: Casamino acid-Peptone-Glucose.
ND: Not identified/Not indicated.
LB: Luria-Bertani.
MEB: Malt extract broth.
PB: Pea broth.
PDA: Potato dextrose agar.
PDB: Potato dextrose broth.
RT: room temperature.
TSB: Trypticase soy broth.

attributed to the presence of many significant compounds, such as ocimene, benzene 1-ethyl-3-methyl- and n-hexadecanoic acid. CFFs from the phytopathogen *A. japonica* have also proven to reduce germination and seedling growth of parthenium (Javaid et al., 2017) and other crop weeds (Dutta et al., 2015) proving that phytotoxins produced by this fungus are not host specific.

However, the use of CFFs in crop protection may also have negative effects, not only as phytotoxic compounds (as noted above) but also on beneficial microorganisms in the agrosystem. In this regard, it has been described how CFFs produced by the pathogen *R. solani* contain coumarin derivatives that significantly reduce mycelial growth of the beneficial fungus *T. harzianum* (Bertagnolli et al., 1998).

The overall data indicate that CFFs from plant-pathogen microorganisms can protect plants by inhibiting growth of pathogenic organisms and enhancing immune response against pathogen attack. In addition, CFFs could fight weeds due to their herbicidal potential. Thus, the application of CFFs can be an environmentally sustainable practice to increase crop health.

3. Way forward and conclusions

Application of cell-free filtrates (CFFs) from plant pathogenic microorganisms on plants is an underexplored field that is being recently investigated. Through this bibliographical review, we conclude that plant pathogen filtrates can be a source of potential benefits for plant health and an alternative to the use of other compounds from living microorganisms, such as those from beneficials. It is evident that the CFFs have an effect on promotion of plant growth and crop health, but different results have been reported depending on the plant and phytopathogen species and its way of obtention and application. These studies are consistent with the recently published by Morcillo et al. (2022) who demonstrated that the application of CFFs of beneficial and phytopathogenic microorganisms is an efficient approach to promote plant growth and improve yield and stress tolerance in a wide range of crops while reducing the use of agrochemicals. Since the filtrate-plant-microorganisms interaction network established seems to be complex, it is necessary to set future research to understand their modes/mechanisms of action in plants.

Indeed, the effectiveness of this strategy will probably expand in the upcoming years as the number of microbial species and strains studied increase. Therefore, comprehensive sampling across various taxonomic levels will be crucial to identify CFFs of agronomical interest. In this context, genetic manipulation of microorganisms provides another unexplored way for enhancing CFFs potential and identifying the biosynthetic pathways involved in the production of their bioactive compounds. This strategy could facilitate the identification of key genes involved in the synthesis of these compounds and would potentially lead to the development of more effective biocontrol agents.

Furthermore, it would be interesting to extend further studies on the implication of these filtrates in unexplored areas, as increasing crop or plant tolerance to extreme weather events (drought, floodings, high CO_2 levels) and other abiotic stresses such as high salinity, heavy metal toxicity and nutrient toxicity or deficiency. Whether *in vitro* or *ex vitro* assays on this field will be essential for understanding the mechanism of action of pathogen cell-free filtrates towards their scientific and commercial potential use as biostimulants and biological control agents in agriculture.

Nevertheless, some limitations exist when it comes to the final product preparation and commercialization, not only for CFFs, but for the biostimulants in general. Some of the remaining challenges include scientific and industrial scale-up studies, formulation stability of the final product and its legal registration and commercialization (Pellegrini et al., 2020).

The principal techniques to obtain high microbial biomass or spores in an industrial scale are submerged liquid and solid-state fermentations. It is well known that these fermentation processes need specific media (carbon and nitrogen sources, phosphate concentration, etc.) and controlled parameters, such as water activity, moisture, inoculum volume, pH, temperature, and control of agitation and aeration of the bioreactor where the microorganism or microorganisms are going to develop (Vassileva et al., 2021; Mattedi et al., 2023). Optimization of physicochemical parameters are the key to achieve maximum product yield and reduce de costs of the final product, but this may be described individually for each type of microorganism.

Recently some studies have successfully validated the upstream process from laboratory to industrial production using *Trichoderma* strains as biostimulant and biocontrol agent (Sala et al., 2021; Modrzewska et al., 2022), some of them also including techno-economic analysis and environmental impact of the pilot-scale scenario (de Lima et al., 2022). Therefore, more exhaustive analyzes are required to allow economic studies of industrial scaling and study if the use of this type of biostimulants is profitable. An economic analysis is fundamental to evaluate the convenience of applying a plant biostimulant. CFFs applications can increase farmers' profitability by improving marketable yield, product quality traits that affect its sale price, or even reducing production cost due to lower input requirements.

The last, but not least, aspect to test before delving into commercialization processes may be final product storage stability, followed by *in vivo* experiments that corroborate their effects on plants. Trujillo-Roldán et al. (2013) carried out a scaling up work for *Azospirillum brasilense*, proving that a product shelf life of up to 2 years could be reached.

Furthermore, and despite their proved potential, biostimulants registration and commercialization is now acting as a bottleneck for the sector development (Vassileva et al., 2021). To now, the European Union is limiting the marketing to products involving microorganisms of the genera *Rhizobium*, *Azotobacter* and *Azospirillum* and mycorrhizal fungi (Regulation (EU) 2019/1009). However, there are many other microorganisms that are currently being used as components of microbial plant biostimulants or are in the research and development phase. In our opinion, breaking all these walls could lead to big steps on a better and sustainable agriculture in the future.

Author contributions

J.P. conceived and designed the manuscript. J.P. wrote the first version of the manuscript. J.P., D.I., Á.M.S.L. and A.F.S.M. performed the bibliographic search, analyzed the information and contributed to the manuscript writing, correction and critical reading. D.I. designed the figures. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

Aldesuquy, H.S., Mansour, F.A., Abo-Hamed, S.A., 1998. Effect of the culture filtrates of *Streptomyces* on growth and productivity of wheat plants. Folia Microbiol. 43, 465–470. https://doi.org/10.1007/BF02820792.

Amari, K., Niehl, A., 2020. Nucleic acid-mediated PAMP-triggered immunity in plants. Curr. Opin. Virol. 42, 32–39. https://doi.org/10.1016/j.coviro.2020.04.003.

Anderson-Prouty, A.J., Albersheim, P., 1975. Host-Pathogen Interactions: VIII. Isolation of a pathogen-synthesized fraction rich in glucan that elicits a defense response in the pathogen's host. Plant Physiol. 56, 286–291. https://doi.org/10.1104/pp.56.2.286.

Ávila, A.C., Poveda, J., 2021. Induction of immune response in *Arabidopsis thaliana* treated with phytopathogen filtrates. Biol. Life Sci. Forum. 11, 85. https://doi.org/10.17632/y3b226n5v3.1.

Ayers, A.R., Ebel, J., Finelli, F., Berger, N., Albersheim, P., 1976. Host-pathogen interactions: IX. Quantitative assays of elicitor activity and characterization of the elicitor present in the extracellular medium of cultures of *Phytophthora megasperma* var. *sojae*. Plant Physiol. 57, 751–759. https://doi.org/10.1104/pp.57.5.751.

Bagde, U.S., Prasad, R., Varma, A., 2011. Influence of culture filtrate of *Piriformospora indica* on growth and yield of seed oil in *Helianthus annus*. Symbiosis 53, 83–88. https://doi.org/10.1007/s13199-011-0114-6.

Bailey, B.A., 1995. Purification of a protein from culture filtrates of *Fusarium oxysporum* that induces ethylene and necrosis in leaves of *Erythroxylum coca*. Phytopathology 85, 1250–1255.

Baroja-Fernandez, E., Goizeder, A., Sánchez-López, Á.M., Abdellatif, B., Gámez-Arcas, S., De Diego, N., Dolezal, K., Muñoz, F.J., Climent-Sanz, E., Pozueta-Romero, J., 2021. Enhanced yield of pepper plants promoted by soil application of volatiles from fungal cultures is associated with activation of the beneficial soil microbiota. Front. Plant Sci. 2396 https://doi.org/10.3389/fpls.2021.752653.

Bertagnolli, B.L., Daly, S., Sinclair, J.B., 1998. Antimycotic compounds from the plant pathogen *Rhizoctonia solani* and its antagonist *Trichoderma harzianum*. J. Phytopathol. (Berl.) 146, 131–135. https://doi.org/10.1111/j.1439-0434.1998.tb04669.x.

Bonnet, P., Rousse, G., 1985. Réactions différentielles du tabac à 9 espèces de *Phytophthora*. Agronomie 5 (9), 801–808. https://doi.org/10.1051/agro:19850905.

Brader, G., Tas, E., Palva, E.T., 2001. Jasmonate-dependent induction of indole glucosinolates in Arabidopsis by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiol. 126, 849–860. https://doi.org/10.1104/pp.126.2.849.

Chang, H.X., Domier, L.L., Radwan, O., Yendrek, C.R., Hudson, M.E., Hartman, G.L., 2016. Identification of multiple phytotoxins produced by *Fusarium virguliforme* including a phytotoxic effector (FvNIS1) associated with sudden death syndrome foliar symptoms. MPMI (Mol. Plant-Microbe Interact.) 29, 96–108. https://doi.org/10.1094/MPMI-09-15-0219-R.

Davis, K., Lyon, G., Darvill, A., Albersheim, P., 1984. Endopolygalacturonic acid lyase from *Erwinia carotovora* elicits phytoalexin accumulation by releasing plant cell wall fragments. Plant Physiol. 74, 52–60. https://doi.org/10.1104/pp.74.1.52.

De Clercq, M., Vats, A., Biel, A., 2018. Agriculture 4.0: the Future of Farming Technology. Proceedings of the World Government Summit, Dubai, UAE, pp. 11–13.

De Lima, E.A., Mandelli, F., Kolling, D., Matsusato Souza, J., de Oliveira Filho, C.A., Ribeiro da Silva, M., Lobo de Mesquita Sampaio, I., Lopes Junqueira, T., Ferreira Chagas, M., Teodoro, J.C., de Morais, E.R., Murakami, M.T., 2022. Development of an economically competitive *Trichoderma*-based platform for enzyme production: bioprocess optimization, pilot plant scale-up, techno-economic analysis and life cycle assessment. Bioresour. Technol. 364, 128019 https://doi.org/10.1016/j.biortech.2022.128019.

dos Santos Lopes, M.J., Dias-Filho, M.B., Gurgel, E.S.C., 2021. Successful plant growth-promoting microbes: inoculation methods and abiotic factors. Front. Sustain. Food Syst. 5, 1–13. https://doi.org/10.3389/fsufs.2021.606454.

Dow, J.M., Callow, J.A., 1979. Partial characterization of glycopeptides from culture filtrates of *Fulvia fulva* (Cooke) ciferri (syn. *Cladosporium fulvum*), the tomato leaf mould pathogen. Microbiology 113, 57–66. https://doi.org/10.1099/00221287-113-1-57.

Dutta, W., Ray, D., Ray, P., 2015. Molecular characterization and host range studies of indigenous fungus as prospective mycoherbicidal agent of water hyacinth. Indian J. Weed Sci. 47, 59–65.

Erikson, D., Montgomery, H.B.S., 1945. Certain aspects of resistance of plum trees to bacterial canker: part III. The action of cell-free filtrates of *Pseudomonas mors-prunorum* Wormald and related phyto-pathogenic bacteria on plum trees. Ann. Appl. Biol. 32, 117–123. https://doi.org/10.1111/j.1744-7348.1945.tb06770.x.

Faris, J.D., Anderson, J.A., Francl, L.J., Jordahl, J.G., 1996. Chromosomal location of a gene conditioning insensitivity in wheat to a necrosis-inducing culture filtrate from *Pyrenophora tritici-repentis*. Phytopathology 86, 459–463.

Fiume, F., Fiume, G., 2003. Use of culture filtrates of *Pyrenochaeta lycopersici* in tests for selecting tolerant varieties of tomato. J. Plant Pathol. 85, 131–133.

Frank, J.A., Paxton, J.D., 1971. An inducer of soybean phytoalexin and its role in the resistance of soybeans to *Phytophthora* rot. Phytopathology 61, 954–958.

Ganesan, M., Jayabalan, N., 2006. Isolation of disease-tolerant cotton (*Gossypium hirsutum* L. cv. SVPR 2) plants by screening somatic embryos with fungal culture filtrate. PCTOC 87, 273–284. https://doi.org/10.1007/s11240-006-9164-5.

Ganugi, P., Martinelli, E., Lucini, L., 2021. Microbial biostimulants as a sustainable approach to improve the functional quality in plant-based foods: a review. Curr. Opin. Food Sci. 41, 217–223. https://doi.org/10.1016/j.cofs.2021.05.001.

Gámez-Arcas, S., Baroja-Fernández, E., García-Gómez, P., Muñoz, F.J., Almagro, G., Bahaji, A., Sánchez-López, Á.M., Pozueta-Romero, J., 2022. Action mechanisms of small microbial volatile compounds in plants. J. Exp. Bot. 73, 498–510. https://doi.org/10.1093/jxb/erab463.

Gentile, A., Tribulato, E., Continella, G., Vardi, A., 1992. Differential responses of citrus calli and protoplasts to culture filtrate and toxin of *Phoma tracheiphila*. Theor. Appl. Genet. 83, 759–764. https://doi.org/10.1007/BF00226695.

Haituk, S., Withee, P., Sangta, J., Senwanna, C., Khamsaw, P., Karunarathna, A., Hongsibsong, S., Sringarm, K., Prasad, S.K., Sommano, S.R., Cheewangkoon, R., 2022. Production of non-volatile metabolites from *Sooty molds* and their bio-functionalities. Processes 10, 329. https://doi.org/10.3390/pr10020329.

Hakim, S., Naqqash, T., Nawaz, M.S., Laraib, I., Siddique, M.J., Zia, R., Mirza, M.S., Imran, A., 2021. Rhizosphere engineering with plant growth-promoting microorganisms for agriculture and ecological sustainability. Front. Sustain. Food Syst. 5, 16. https://doi.org/10.3389/fsufs.2021.617157.

Hassine, M., Aydi-Ben-Abdallah, R., Jabnoun-Khireddine, H., Daami-Remadi, M., 2022. Soil-borne and compost-borne *Penicillium* sp. and *Gliocladium* spp. as potential microbial biocontrol agents for the suppression of anthracnose-induced decay on tomato fruits. Egypt J. Biol. Pest Control 32 (1), 20. https://doi.org/10.1186/s41938-022-00519-5.

Healy, F.G., Wach, M., Krasnoff, S.B., Gibson, D.M., Loria, R., 2000. The *txtAB* genes of the plant pathogen *Streptomyces acidiscabies* encode a peptide synthetase required for phytotoxin thaxtomin A production and pathogenicity. Mol. Microbiol. 38, 794–804. https://doi.org/10.1046/j.1365-2958.2000.02170.x.

Huet, J.-C., Nespoulous, C., Pernollet, J.-C., 1992. Structures of elicitin isoforms secreted by *Phytophthora drechsleri*. Phytochemistry 31 (5), 1471–1476. https://doi.org/10.1016/0031-9422(92)83089-H.

Inbar, J., Chet, I., 1994. A newly isolated lectin from the plant pathogenic fungus *Sclerotium roltsii:* purification, characterization and role in mycoparasitism. Microbiology 140, 651–657. https://doi.org/10.1099/00221287-140-3-651.

Javaid, A., Mubeen, T., Bashir, U., Shoaib, A., 2017. Management of parthenium weed by metabolites of *Alternaria japonica*. Planta Daninha 35, e017161195. https://doi.org/10.1590/S0100-83582017350100016.

Jayasankar, S., Li, Z., Gray, D.J., 2000. In-vitro selection of *Vitis vinifera* Chardonnay with *Elsinoe ampelina* culture filtrate is accompanied by fungal resistance and enhanced secretion of chitinase. Planta 211, 200–208. https://doi.org/10.1007/s004250000285.

Jhariya, M.K., Meena, R.S., Banerjee, A., 2021. Ecological intensification of natural resources towards sustainable productive system. In: Ecological Intensification of Natural Resources for Sustainable Agriculture. Springer, pp. 1–28. https://doi.org/10.1007/978-981-33-4203-3_1.

Kaur, T., Rani, R., Manhas, R.K., 2019. Biocontrol and plant growth promoting potential of phylogenetically new *Streptomyces* sp. MR14 of rhizospheric origin. Amb. Express 9, 125. https://doi.org/10.1186/s13568-019-0849-7.

Kausar, T., Jabeen, K., Javaid, A., Iqbal, S., 2022. Herbicidal efficacy of culture filtrates of *Alternaria brassicicola* and *Alternaria gaisen* against parthenium weed. Adv Weed Sci 40. https://doi.org/10.51694/AdvWeedSci/2022;40:00002.

Khan, N., Bano, A., Rahman, M.A., Guo, J., Kang, Z., Babar, M.A., 2019. Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in Chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. Sci. Rep. 9, 2097. https://doi.org/10.1038/s41598-019-38702-8.

Mattedi, A., Sabbi, E., Farda, B., Djebaili, R., Mitra, D., Ercole, C., Cacchio, P., Del Gallo, M., Pellegrini, M., 2023. Solid-state fermentation: applications and future perspectives for biostimulant and biopesticides production. Microorganisms 11 (6). https://doi.org/10.3390/microorganisms11061408.

Martínez-Viveros, O., Jorquera, M.A., Crowley, D.E., Gajardo, G., Mora, M.L., 2010. Mechanisms and practical considerations involved in plant growth promotion by Rhizobacteria. J. Soil Sci. Plant Nutr. 10, 293–319. https://doi.org/10.4067/S0718-95162010000100006.

Mathivanan, N., Prabavathy, V.R., Vijayanandraj, V.R., 2008. The effect of fungal secondary metabolites on bacterial and fungal pathogens. Secondary Metabolites in Soil Ecology 14, 129–140. https://doi.org/10.1007/978-3-540-74543-3_7.

McLellan, H., Boevink, P.C., Armstrong, M.R., Pritchard, L., Gomez, S., Morales, J., Whisson, S.C., Beynom, J.L., Birch, P.R., 2013. An RxLR effector from *Phytophthora infestans* prevents re-localisation of two plant NAC transcription factors from the endoplasmic reticulum to the nucleus. PLoS Pathog. 9, e1003670 https://doi.org/10.1371/journal.ppat.1003670.

Meena, M., Swapnil, P., Zehra, A., Dubey, M.K., Upadhyay, R.S., 2017. Antagonistic assessment of *Trichoderma* spp. by producing volatile and non-volatile compounds against different fungal pathogens. Arch. Phytopathol. 50, 629–648. https://doi.org/10.1080/03235408.2017.1357360.

Modrzewska, M., Bryła, M., Kanabus, J., Pierzgalski, A., 2022. *Trichoderma* as a biostimulator and biocontrol agent against *Fusarium* in the production of cereal crops: opportunities and possibilities. Plant Pathol. 71, 1471–1485. https://doi.org/10.1111/ppa.13578.

Mohammad-Razdari, A., Rousseau, D., Bakhshipour, A., Taylor, S., Poveda, J., Kiani, H., 2022. Recent advances in E-monitoring of plant diseases. Biosens. Bioelectron. 21, 2129. https://doi.org/10.1016/j.bios.2021.113953.

Monteiro, A., Santos, S., 2022. Sustainable approach to weed management: the role of precision weed management. Agronomy 12 (1). https://doi.org/10.3390/agronomy12010118. Article 1.

Morcillo, R.J.L., Baroja-Fernández, E., López-Serrano, L., Leal-López, J., Muñoz, F.J., Bahaji, A., Férez-Gómez, A., Pozueta-Romero, J., 2022. Cell-free microbial culture filtrates as candidate biostimulants to enhance plant growth and yield and activate soil-and plant-associated beneficial microbiota. Front. Plant Sci. 13 (2022), 5325. https://doi.org/10.3389/fpls.2022.1040515.

Naamala, J., Smith, D.L., 2021. Microbial derived compounds, a step toward enhancing microbial inoculants technology for sustainable agriculture. Front. Microbiol. 12, 1–12. https://doi.org/10.3389/fmicb.2021.634807.

Nguyen, Q.M., Iswanto, A.B.B., Son, G.H., Kim, S.H., 2021. Recent advances in effector-triggered immunity in plants: new pieces in the puzzle create a different paradigm. Int. J. Mol. Sci. 22, 4709. https://doi.org/10.3390/ijms22094709.

Oerke, E.-C., 2006. Crop losses to pests. J. Agric. Sci. 144, 31–43. https://doi.org/10.1017/S0021859605005708.

Ogórek, R., 2016. Enzymatic activity of potential fungal plant pathogens and the effect of their culture filtrates on seed germination and seedling growth of garden cress (*Lepidium sativum* l.). Eur. J. Plant Pathol. 145 (2), 469–481. https://doi.org/10.1007/s10658-016-0860-7.

Orsomando, G., Lorenzi, M., Raffaelli, N., Dalla Rizza, M., Mezzetti, B., Ruggieri, S., 2001. Phytotoxic protein PcF, purification, characterization, and cDNA sequencing of a novel hydroxyproline-containing factor secreted by the strawberry pathogen *Phytophthora cactorum*. J. Biol. Chem. 276, 21578–21584. https://doi.org/10.1074/jbc.M101377200.

Pacios-Michelena, S., González, C.N.A., Herrera, R.R., Alvarez-Perez, O.B., González, M. L.C., Valdés, R.A., Valdés, J.A.A., Salas, M.G., Iliná, A., 2023. Biomass from phytopathogens and culture conditions improve *Penicillium chrysogenum* antimicrobial activity and antifungal compounds production. Environ. Qual. Manag. 33 (1), 349–358. https://doi.org/10.1002/tqem.22065.

Parveen, S., Wani, A.H., Bhat, M.Y., 2019. Effect of culture filtrates of pathogenic and antagonistic fungi on seed germination of some economically important vegetables. Braz. J. Biol. Sci. 6, 133–139. https://doi.org/10.21472/bjbs.061212.

Paul, A., Sarkar, A., Acharya, K., Chakraborty, N., 2022. Fungal elicitor-mediated induction of innate immunity in *Catharanthus roseus* against leaf blight disease caused by *Alternaria alternata*. J. Plant Growth Regul. 42 https://doi.org/10.1007/s00344-021-10569-y.

Pawlak, K., Kołodziejczak, M., 2020. The role of agriculture in ensuring food security in developing countries: considerations in the context of the problem of sustainable food production. Sustainability 12, 5488. https://doi.org/10.3390/su12135488.

Prasad, M., Srinivasan, R., Chaudhary, M., Choudhary, M., Jat, L.K., 2019. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture: perspectives and challenges. In: PGPR Amelioration in Sustainable Agriculture. Woodhead Publishing, pp. 129–157. https://doi.org/10.1016/B978-0-12-815879-1.00007-0.

Pellegrini, M., Pagnani, G., Bernardi, M., Mattedi, A., Spera, D.M., Gallo, M.D., 2020. Cell-free supernatants of plant growth-promoting bacteria: areview of their use as biostimulant and microbial biocontrol agents in sustainable agriculture. Sustainability 12, 9917. https://doi.org/10.3390/su12239917.

Pfund, C., Tans-Kersten, J., Dunning, F.M., Alonso, J.M., Ecker, J.R., Allen, C., Bent, A.F., 2004. Flagellin is not a major defense elicitor in *Ralstonia solanacearum* cells or extracts applied to *Arabidopsis thaliana*. MPMI (Mol. Plant-Microbe Interact.) 17, 696–706. https://doi.org/10.1094/MPMI.2004.17.6.696.

Pirttilä, A.M., Mohammad Parast Tabas, H., Baruah, N., Koskimäki, J.J., 2021. Biofertilizers and biocontrol agents for agriculture: how to identify and develop new potent microbial strains and traits. Microorganisms 9, 817. https://doi.org/10.3390/microorganisms9040817.

Ponce de León, I., Oliver, J.P., Castro, A., Gaggero, C., Bentancor, M., Vidal, S., 2007. *Erwinia carotovora* elicitors and *Botrytis cinerea* activate defense responses in *Physcomitrella patens*. BMC Plant Biol. 7, 1–11. https://doi.org/10.1186/1471-2229-7-52.

Poveda, J., 2020. Use of plant-defense hormones against pathogen-diseases of postharvest fresh produce. Physiol. Mol. Plant Pathol. 111, 101521 https://doi.org/10.1016/j.pmpp.2020.101521.

Poveda, J., 2021a. Trichoderma as biocontrol agent against pests: new uses for a mycoparasite. Biol. Control 159, 104634. https://doi.org/10.1016/j.biocontrol.2021.104634.

Poveda, J., 2021b. Beneficial effects of microbial volatile organic compounds (MVOCs) in plants. Appl. Soil Ecol. 168, 104118 https://doi.org/10.1016/j.apsoil.2021.104118.

Poveda, J., González-Andrés, F., 2021. *Bacillus* as a source of phytohormones for use in agriculture. Appl. Microbiol. Biotechnol. 105, 8629–8645. https://doi.org/10.1007/s00253-021-11492-8.

Poveda, J., 2022. Effect of volatile and non-volatile metabolites from *Leptosphaeria maculans* on tomato calli under abiotic stresses. Plant Stress 3, 100054. https://doi.org/10.1016/j.stress.2021.100054.

Rahman, A., Sultana, R., Ferdousi Begum, M., Firoz Alam, M., Asaduzzaman, M., Sultana, R., Ferdousi Begum, M., Firoz Alam, M., 2012. Effect of culture filtrate of *Trichoderma* on seed germination and seedling growth in chili. Int. J. Biosci. 2, 46–55. https://doi.org/10.3329/jsf.v8i1-2.14637.

Regulation (EU) 2019/1009, 2019. European Parliament and of the Council of 5 June 2019 Laying Down Rules on the Making Available on the Market of EU Fertilising Products and Amending Regulations. of the. In: (EC) No 1069/2009 and (EC) No 1107/2009 and Repealing Regulation (EC) No 2003/2003 (Text with EEA Relevance).

Remick, B.C., Gaidt, M.M., Vance, R.E., 2023. Effector-triggered immunity. Annu. Rev. Immunol. 41, 453–481. https://doi.org/10.1146/annurev-immunol-101721-031732.

Rose, D.C., Chilvers, J., 2018. Agriculture 4.0: broadening responsible innovation in an era of smart farming. Front. Sustain. Food Syst. 2, 87. https://doi.org/10.3389/fsufs.2018.00087.

Rowe, S.L., Norman, J.S., Friesen, M.L., 2018. Coercion in the evolution of plant–microbe communication: a perspective. MPMI (Mol. Plant-Microbe Interact.) 31, 789–794. https://doi.org/10.1094/MPMI-11-17-0276-CR.

Sala, A., Barrena, R., Sánchez, A., Artola, A., 2021. Fungal biopesticide production: process scale-up and sequential batch mode operation with *Trichoderma harzianum* using agro-industrial solid wastes of different biodegradability. Chem. Eng. J. 425, 131620 https://doi.org/10.1016/j.cej.2021.131620.

Shahzad, A., Ullah, S., Dar, A.A., Sardar, M.F., Mehmood, T., Tufail, M.A., Shakoor, A., Haris, M., 2021. Nexus on climate change: agriculture and possible solution to cope future climate change stresses. ESPR 28, 14211–14232. https://doi.org/10.1007/s11356-021-12649-8.

Sharma, S., Kaur, M., 2017. Plant hormones synthesized by microorganisms and their role in biofertilizer. IJAR 5, 1753–1766. https://doi.org/10.21474/ijar01/6144.

Singh, U.P., Birinchi, K.S., Singh, D.P., 2003. Effect of plant growth-promoting rhizobacteria and culture filtrate of *Sclerotium rolfsii* on phenolic and salicylic acid contents in chickpea (*Cicer Arietinum*). Curr. Microbiol. 46, 131–140. https://doi.org/10.1007/s00284-002-3834-2.

Singh, M., Bhasin, S., Madan, N., Suyal, D.C., Soni, R., Singh, D., 2021. Bioinoculants for agricultural sustainability. In: Microbiological Activity for Soil and Plant Health Management. Springer, pp. 629–641. https://doi.org/10.1007/978-981-16-2922-8_25.

Skendžić, S., Zovko, M., Živković, I.P., Lešić, V., Lemić, D., 2021. The impact of climate change on agricultural insect pests. Insects 12, 440. https://doi.org/10.3390/insects12050440.

Strange, R.N., 2007. Phytotoxins produced by microbial plant pathogens. Nat. Prod. Rep. 24, 127–144. https://doi.org/10.1039/b513232k.

Sung, G.H., Shrestha, B., Park, K.B., Han, S.K., Sung, J.M., 2011. Enhancing effect of *Shimizuomyces paradoxus* on seed germination and seedling growth of canola, plant growth of cucumber, and harvest of tomato. MYCOBIOLOGY 39, 7–11. https://doi.org/10.4489/MYCO.2011.39.1.007.

Švábová, L., Lebeda, A., 2005. In vitro selection for improved plant resistance to toxin-producing pathogens. J. Phytopathol. 153, 52–64. https://doi.org/10.1111/j.1439-0434.2004.00928.x.

Tomas, A., Bockus, W.W., 1987. Cultivar-specific toxicity of culture filtrates of *Pyrenophora tritici-repentis*. Phytopathology 77, 1337–1340.

Tomas-Grau, R.H., Hael-Conrad, V., Requena-Serra, F.J., Perato, S.M., Caro, M.D.P., Salazar, S.M., Díaz-Ricci, J.C., 2020. Biological control of strawberry grey mold disease caused by *Botrytis cinerea* mediated by *Colletotrichum acutatum* extracts. Biocontrol 65, 461–473. https://doi.org/10.1007/s10526-020-10003-4.

Trujillo-Roldán, M.A., Valdez-Cruz, N.A., Gonzalez-Monterrubio, C.F., Acevedo-Sánchez, E.V., Martínez-Salinas, C., García-Cabrera, R.I., Gamboa-Suasnavart, R.A., Marín-Palacio, L.D., Villegas, J., Blancas-Cabrera, A., 2013. Scale-up from shake flasks to pilot-scale production of the plant growth-promoting bacterium *Azospirillum brasilense* for preparing a liquid inoculant formulation. Appl. Microbiol. Biotechnol. 97, 9665–9674. https://doi.org/10.1007/s00253-013-5199-9.

Tsuge, T., Harimoto, Y., Akimitsu, K., Ohtani, K., Kodama, M., Akagi, Y., Egusa, M., Yamamoto, M., Otani, H., 2013. Host-selective toxins produced by the plant pathogenic fungus *Alternaria alternata*. FEMS Microbiol. Rev. 37, 44–66. https://doi.org/10.1111/j.1574-6976.2012.00350.x.

Tudi, M., Daniel Ruan, H., Wang, L., Lyu, J., Sadler, R., Connell, D., Chu, C., Phung, D.T., 2021. Agriculture development, pesticide application and its impact on the environment. Int. J. Environ. Res. Publ. Health 18, 1112. https://doi.org/10.3390/ijerph18031112.

Varma, A., Savita, V., Sudha, Sahay, N., Butehorn, B., Franken, P., 1999. *Piriformospora indica*, a cultivable plant-growth-promoting root endophyte. Appl. Environ. Microbiol. 65, 2741–2744. https://doi.org/10.1128/AEM.65.6.2741-2744.1999.

Vassileva, M., Malusà, E., Sas-Paszt, L., Trzcinski, P., Galvez, A., Flor-Peregrin, E., Shilev, S., Canfora, L., Mocali, S., Vassilev, N., 2021. Fermentation strategies to improve soil bio-inoculant production and quality. Microorganisms 9, 1254. https://doi.org/10.3390/microorganisms9061254.

Vidal, S., Eriksson, A.R., Montesano, M., Denecke, J., Palva, E.T., 1998. Cell wall-degrading enzymes from *Erwinia carotovora* cooperate in the salicylic acid-independent induction of a plant defense response. MPMI (Mol. Plant-Microbe Interact.) 11, 23–32. https://doi.org/10.1094/MPMI.1998.11.1.23.

Vijaya-Kumar, J., Ranjitha-Kumari, B.D., Sujatha, G., Castano, E., 2008. Production of plants resistant to *Alternaria carthami* via organogenesis and somatic embryogenesis of safflower cv. NARI-6 treated with fungal culture filtrates. PCTOC 93, 85–96. https://doi.org/10.1007/s11240-008-9346-4.

Wang, H., Fu, L., Meng, J., Ding, R., Wang, Y., Wang, X., Han, C., Li, L., Zhu, C., 2022. Antagonistic activity and biocontrol effects of *Streptomyces* sp. CX3 cell-free supernatant against blueberry canker caused by *Botryosphaeria dothidea*. Crop Protect. 162, 106072 https://doi.org/10.1016/j.cropro.2022.106072.

Wilson, L.M., Idnurm, A., Howlett, B.J., 2002. Characterization of a gene (*sp1*) encoding a secreted protein from *Leptosphaeria maculans*, the blackleg pathogen of *Brassica napus*. Mol. Plant Pathol. 3, 487–493. https://doi.org/10.1046/j.1364-3703.2002.00144.x.

Wu, L., Wu, H., Chen, L., Yu, X., Borriss, R., Gao, X., 2015. Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. Sci. Rep. 5, 1–9. https://doi.org/10.1038/srep12975.

Yadav, A.N., 2021. Beneficial plant-microbe interactions for agricultural sustainability. J. Appl. Biol. Biotechnol. 9, 1–4. https://doi.org/10.7324/JABB.2021.91ed.

Yandigeri, M.S., Meena, K.K., Singh, D., Malviya, N., Singh, D.P., Solanki, M.K., Yadav, A. K., Arora, D.K., 2012. Drought-tolerant endophytic actinobacteria promote growth of wheat (*Triticum aestivum*) under water stress conditions. Plant Growth Regul. 68, 411–420. https://doi.org/10.1007/s10725-012-9730-2.