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In Vitro Fungistatic Bioactivity of a Biostimulant Based on Pine Bark Extract Against Phytopathogenic Fungi

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Abstract

The use of biostimulants and corroborants is increasing worldwide. Laboratory and field assays show their effectiveness in improving the vegetative performance of plants and their tolerance to abiotic stresses. This study aims to evaluate the *in vitro* activity of a biostimulant, based on pine bark extract, against some fungal phytopathogens. This research was carried out at the Laboratory of Plant Pathology (SAAF Department, University of Palermo, Italy), employing the poison food technique. Artificial agar media (Potato Dextrose Agar, PDA), simple or added with different concentrations of the biostimulant, were used to evaluate the differences in diametral growth of the fungi *Aspergillus niger*, *Aspergillus tubingensis*, *Botrytis cinerea*, *Coriolopsis gallica*, *Fomitiporia mediterranea*, *Fusarium oxysporum*, *Pleurostoma richardsiae* and *Pleurotus ostreatus*. The biostimulant was shown to contain the growth of most of the tested fungi, with the greatest effectiveness on *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae* at the highest concentration, moderate effects on *A. niger*, *F. oxysporum* and *P. ostreatus* and no effect on *B. cinerea*. The observed fungistatic effects suggest that this biostimulant could contribute to integrated disease management while supporting more sustainable crop protection practices. In vivo tests aimed at evaluating the efficacy of these products on the evolution of different diseases in the field are ongoing, and preliminary results are promising but they are part of future work.

Keywords: biostimulants; bioactivity; phytopathogenic fungi; poisoned media

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1. Introduction

Traditionally, the amelioration of vegetative performance and plant protection is pursued through the employment of highly efficient chemical products of natural or synthetical origin. However, researchers have raised concerns about their negative impact on the environment, biodiversity and human health. To overcome this gap, natural products have been developed to improve growth, production and tolerance/resistance against abiotic and biotic stresses, reducing the environmental and sanitary risks. In recent years, the use of these products on plants has received increasing attention, being eco-compatible, readily available, inexpensive and renewable sources [1]. Most of them, known as biostimulants and corroborants (plant strengtheners), activate the host physiological mechanisms that induce beneficial effects on the whole plant by implementing natural defenses against parasites of various kinds [2].

Biostimulants promote the nutritional processes of plants, independently of the nutrient content of the formulate and improve both the tolerance to abiotic stress and the availability of nutrients contained in the soil or rhizosphere (https://eur-lex.europa.eu/eli/reg/2019/1009/oj accessed on 13 February 2024). Associations such as the European Biostimulants Industry Council (EBIC, founded in 2011) or the Biostimulant Coalition (in the USA, also founded in 2011) aim to define the concept and the diffusion of biostimulants, linking industries and the world of scientific research to ensure an adequate regulatory framework for a clear vision of biostimulant products. In particular, the EBIC defines a biostimulant as "a material which contains substance(s) and/or microorganisms whose function, when applied to plants or the rhizosphere, is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality, independent of its nutrient content" (EBIC, biostimolants.eu, 2019, https://biostimulants.eu/plant-biostimulants/; accessed on 20 February 2024).

Corroborants, instead, are defined as a substance of natural origin that improves and increases the natural resistance of plants against harmful organisms and abiotic damage. They can also encourage the secondary metabolism of the plant to contain attacks by pathogens and parasites by acting as "physical insulating systems". According to this definition, "harmful organisms" are "any species, strain or biotype belonging to the animal or plant kingdom, as well as other pathogenic agents harmful to plants or plant products" (Decree no. 6793/2018 of the Italian Ministry of Agricultural, Food, Forestry and Tourism Policies https://www.gazzettaufficiale.it/eli/id/2020/06/22/20A03222/sg accessed on 18 May 2025).

Based on these guidelines, biostimulants and corroborants are not considered as pesticides. However, despite this, the latest studies have ascertained the bioactivity of some of these products against phytopathogens and insect pests (Table 1).

Table 1. Bioactivity of some biostimulants based on natural extracts against phytopathogens and insect pests.

Microorganisms Tested	Type of Product	Bibliographic Source
Bacteria		
Agrobacterium tumefaciens, *A. vitis*, *Clavibacter michiganensis* subsp. *michiganensis*, *Erwinia amylovora*, *E. carotovora* pv. carotovora, *Pseudomonas corrugata*, *P. savastanoi* pv. *savastanoi*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *syringae*, *P. syringae* pv. tomato, *Ralstonia solanacearum*, *Xanthomonas campestris* pv. *Campestris*, *X. axonopodis* pv. *vesicatoria*.	Turkish pollen and propolis extracts	[3]
Escherichia coli, *Staphylococcus aureus*	Seaweed extract	[3]
P. syringae pv. *tomato*	Tannins	[4]
E. coli, *Salmonela thyphei*, *S. aureus*, *Streptococcus agalactiae*	Leaf extract of *Plantago lanceolata*	[5]
Fungi		
Lenzites trabea	Bark extracts	[6]
Penicillium italicum	Propolis	[7]
Alternaria alternata, *Fusarium* sp., *Botrytis cinerea*, *P. expansum*, *Trichoderma reesei*, *Ulocladium* sp.	Chilean propolis	[8]
Colletotrichum gloeosporioides	Propolis	[9]
Coniophera puteana, *Trametes versicolor*	Wood bark extracts	[10]
A. niger and *Fusarium solani*	Leaf extract of *Plantago lanceolata*	[5]
T. versicolor, *C. puteana*	Gray alder bark extracts	[11]
Phytopathogenic fungi	Propolis	[12]
Alternaria sp., *Rhizoctonia* sp., *Fusarium* sp.	Grapefruit extract, tea tree oil and *Trichoderma harzianum* T-22	[13]
Fusarium spp.	Plant essential oils and propolis	[14]

Table 1. *Cont.*

Microorganisms Tested	Type of Product	Bibliographic Source
Several plant pathogens		
	Chitin and chitosan	[15]
	Resin acid derivates	[16]
	Moringa oleifera extract	[17]
	Chitin and chitosan	[18]
Insects		
Reculitermes flavipes	Bark extracts	[6]

Regarding the distinction of biostimulant products in categories, the first important classification was proposed by du Jardin [19], who divides these substances into seven categories: humic and fulvic acids; protein hydrolysates and other nitrogen-containing compounds; seaweed extracts and botanicals; chitosan and other biopolymers; inorganic compounds; beneficial fungi; and beneficial bacteria. To these categories, Shahrajabian et al. [15] also added the following: phosphites; silicon; and vermicompost. Researchers have tried to classify compounds and substances with biostimulant activity, but categorizing them according to the mechanism of action is also particularly difficult due to their interaction with a wide range of other biological molecules [20].

Among the different natural substances and products employed as biostimulants, the pine bark extract is considered one of the most active. Several studies indicate it is a potential source of antioxidants and other bioactive compounds. Polymeric flavanols, tannic acid, flavonoids and other phenolic acids such as caffeic or protocatechuic acid have been shown to be useful in treating diseases [21], or for biological, nutraceutical and clinical aspects [22]. Biological activity of pine bark in food and the pharmaceutical industries [23] and its antioxidant or antibacterial activities [24] have been experimentally demonstrated. At the same time, pine bark extracts and pine bark powders were evaluated *in vitro* and in greenhouse experiments for agricultural purposes, for example, their effect on fungal growth and infectivity, soil enzyme activity and soil microbial populations [25].

Based on these considerations, *in vitro* tests were conducted at the Laboratory of Plant Pathology of the Department of Agriculture, Food and Forest Sciences (SAAF) of the University of Palermo to evaluate the bioactivity of a pine bark biostimulant (produced by SavorySun VA LLC, Fredericksburg, VA, USA) against fungal phytopathogen agents of various aetiologies. Aside from the various benefits of pine extract listed above, this pine bark biostimulant can be easily produced by the company. It is obtained from systems that do not use chemical formulations, and it can be obtained in large quantities starting from a waste product.

2. Materials and Methods

2.1. In Vitro Tests

Fungal Microorganisms and Product Used

The study was conducted at the Laboratory of Plant pathology of the Department of Agricultural, Food and Forest Sciences of the University of Palermo (Italy) from the end of 2022 until January 2024. In this work a pine bark biostimulant was tested against some fungal pathogens, isolated from different diseased hosts (Table 2). The main pine components contained in this biostimulant are pine oil 85, oleoresin, α-terpineol, gum turpentine and dipentene. The pine extracts were obtained via steam distillation through a packed or bubble-capped column using 50% ethanol as the extraction solvent.

Table 2. Fungal microorganisms tested.

Fungal Microorganisms	GenBank Accession Number	Type of Damage	Isolated from
Coriolopsis gallica (Fr.) Ryvarden	OP028965	White wood rot	*Olea europaea* L.
Botrytis cinerea (Pers.)	KM265453.1	Soft rot	*Vitis vinifera* L. grape
Fomitiporia mediterranea M. Fisch	OP028964	White wood rot	*Olea europaea* L.
Fusarium oxysporum Schlechtendal: Fries.	JQ693405.1	Tracheomycosis	*Fragaria vesca* L.
Pleurostoma richardsiae (Nannf.) Reblova and Jaklitsch	OP028963	Tracheomycosis	*Olea europaea* L.
Pleurotus ostreatus (Jacq.:Fr.) P. Kumm	OP346051.1	White wood rot	*Ficus macrophylla* Pers. subsp. *Columnaris* (C. Moore)
Aspergillus tubingensis Mosseray	MK503965	Ochratoxigenic	Compound feed for cattle
Aspergillus niger van Tieghem	MK503966	Ochratoxigenic	Compound feed for horses

2.2. Preparation of Substrates

To evaluate the antagonistic activity of the pine bark biostimulant against phytopathogenic fungi, *in vitro* antagonism assays were conducted according to the agar dilution method [26] and to the poison food technique [12,27,28], employing PDA (Potato Dextrose Agar, Oxoid, UK) as the artificial culture medium.

For this work, three concentrations of the biostimulant were used, based on commercial recommended rates and preliminary dose–response trials (different growth of fungal pathogens based on concentration). The pine-based biostimulant is a formulation that can be diluted in water; for this reason, no solvent was used for the control. In particular, the concentration of the biostimulant provided by the producer, a half and a tenth (12, 6, 1.2 $mL \cdot l^{-1}$) were added to the substrate composed of water and PDA before autoclaving. The substrate was then sterilely poured into Petri dishes with a diameter of 9 cm.

2.3. Fungal Growth

All the fungal microorganisms, maintained in a collection at the mycotheca of the Plant Pathology laboratories of the SAAF Department, were grown on PDA in Petri dishes (diameter of 9 cm) and incubated for 6 days at 22 °C. After the incubation period, for each colony, plugs (5 mm in diameter) were taken with a hole punch and placed in the center of each poisoned and un-poisoned (control) substrate. For each pathogen tested, 3 replicates were carried out. The plates were sealed with parafilm and incubated in a thermostatic incubator (22 °C).

2.4. Valuation of the Growth Inhibition

Every 3 days for 9 days, the diametral growth of fungal colonies was detected by measuring two diagonals (in cm) from below the Petri dish. Once the data were collected, it was possible to evaluate for each fungal strain the growth inhibition on the different substrates compared to the control (PDA) using the formula used by Nduagu et al. [28]:

$$\text{Growth inhibition } Mr = \left(\frac{M1 - M2}{M1}\right) \times 100$$

where Mr = reduction in % of the diameter of the fungal mycelium, M1 = growth of the fungal mycelium in the control (cm) and M2 = growth of the fungal mycelium on substrate with PDA + biostimulant (cm).

2.5. Statistical Analysis

The data for mycelial growth (colony diameter) were analyzed using a two-way ANOVA with the replicates to assess the effects of two independent factors: culture medium type (PDA and PDA + biostimulant) and fungal colony tested (8 different fungal colonies). Eight fungal species were tested under four treatment conditions (PDA and three concentrations of the pine bark biostimulant), each with three replicates. This resulted in a total of 24 observations per treatment group and 96 observations overall. The analysis was performed considering the interaction between the two factors. Replicates were included in the model to account for variability between the repeated observations. A normality test on the residuals was performed to check the assumption of normal distribution, while homogeneity of variance was assessed using Levene's test. The significance of the main effects and the interaction was evaluated at a significance level of $\alpha = 0.05$. In the case of significant effects, Tukey's post hoc test was performed to compare the means between the groups. All analyses were performed using Minitab 19 software.

3. Results

The investigations conducted highlighted different levels of bioactivity against the fungal pathogens tested.

Compared to colonies grown on PDA, the biostimulant at the higher concentration (12 mL L^{-1}) was shown to totally inhibit the mycelial growth of *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae* (Figure 1). A good level of bioactivity was detected for *A. niger*, *F. oxysporum* and *P. ostreatus* and no mycelial reduction was observed for *B. cinerea* (Figure 1).

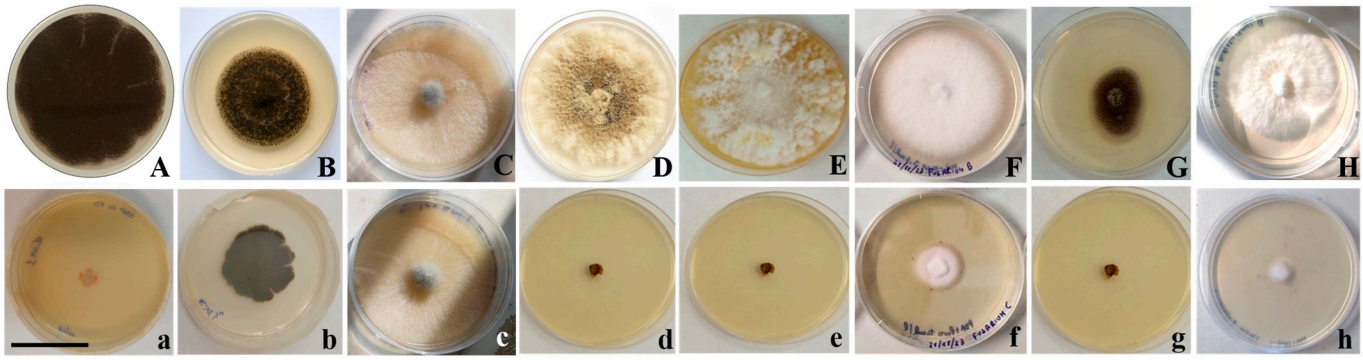

Figure 1. Mycelial growth on PDA culture medium (**A**–**H**) and on culture medium added with biostimulant (concentration of 12 mL L^{-1}; (**a**–**h**)) of different fungal pathogens: *A. tubigensis* PDA (**A**) vs. PDA + biostimulant (**a**); *A. niger* PDA (**B**) vs. PDA + biostimulant (**b**); *B. cinerea* PDA (**C**) vs. PDA + biostimulant (**c**); *C. gallica* PDA (**D**) vs. PDA + biostimulant (**d**); *F. mediterranea* PDA (**E**) vs. PDA + biostimulant (**e**); *F. oxysporum* PDA (**F**) vs. PDA + biostimulant (**f**); *P. richardsiae* PDA (**G**) vs. PDA + biostimulant (**g**); and *P. ostreatus* PDA (**H**) vs. PDA + biostimulant (**h**). Scale bar = 5 cm.

The analysis of variance (ANOVA) on the diametral mycelial growth at the three different concentrations of the biostimulant showed statistically significant differences. The analysis showed significant differences both for the fungal colony tested (df = 7, p-value < 0.001, F = 412.04) and the different substrates employed (df = 3, p-value < 0.001, F = 685.71). Regarding the fungal colony tested, the Tukey's test results showed significant differences in their development for five groups (Table 3): (A) *P. richardsiae* showed the lowest growth among the tested fungal colonies (2.2 cm); (B) *F. mediterranea* and *P. ostreatus* had similar, but significantly higher, growth compared to the fungi in group A (ranging from 2.73 to 2.92 cm); (C) *A. tubingensis* with average values of 4; (D) *A. niger*, *C. gallica* and *F. oxysporum* with values of growth greater than 5.00; and (E) *B. cinerea* exhibited the

greatest growth with an average of 9.00. Regarding the statistical analysis conducted on the culture medium type, the results of Tukey's test showed that there are four groups statistically different from each other: PDA (higher growth of 7.02 cm); PDA + biostimulant 1.2 mL L^{-1} (values around 5 cm); PDA + biostimulant 6 mL L^{-1} (intermediate values, 4.69 cm); and PDA + biostimulant 12 mL L^{-1} (values lower than 2.2 cm).

Table 3. Mycelial growth and mycelial reduction in the fungal colonies tested.

Fungal Species Tested	Concentration of Biostimulant [mL·L^{-1}]	Mycelial Growth [cm]	Mycelial Reduction [%]
A. niger	PDA	9.00 ± 0.000 a	-
	12	3.56 ± 0.549 hij	60.37
	6	4.78 ± 0.072 defg	46.85
	1.2	5.61 ± 0.176 cde	35.79
A. tubingensis	PDA	9.00 ± 0.000 a	-
	12	0.05 ± 0.000 kl	**100**
	6	3.43 ± 0.076 hij	61.85
	1.2	4.60 ± 0.419 efgh	48.89
B. cinerea	PDA	9.00 ± 0.000 a	-
	12	9.00 ± 0.000 a	0.00
	6	9.00 ± 0.000 a	0.00
	1.2	9.00 ± 0.000 a	0.00
C. gallica	PDA	7.55 ± 0.306 b	-
	12	0.05 ± 0.000 l	**100**
	6	6.80 ± 0.161 bc	9.93
	1.2	7.62 ± 0.133 b	−0.88
F. mediterranea	PDA	4.02 ± 0.092 ghij	-
	12	0.05 ± 0.000 l	**100**
	6	3.55 ± 0.208 hij	11.62
	1.2	4.10 ± 0.050 fghi	−2.07
F. oxysporum	PDA	7.52 ± 0.509 b	-
	12	2.92 ± 0.040 ij	61.20
	6	5.85 ± 0.486 cd	22.17
	1.2	5.25 ± 0.425 def	30.16
P. ostreatus	PDA	7.25 ± 0.202 b	-
	12	1.00 ± 0.050 kl	86.21
	6	1.23 ± 0.016 kl	82.99
	1.2	1.45 ± 0.161 k	80.00
P. richardsiae	PDA	2.87 ± 0.130 j	-
	12	0.05 ± 0.000 l	**100**
	6	2.92 ± 0.054 ij	−1.45
	1.2	3.07 ± 0.169 ij	−6.98

Different letters mean significant statistical differences and negative % values indicate stimulation.

The Tukey's test results for the interaction between different culture media and fungal colonies tested showed significant differences (df = 21, p-value < 0.001, F = 53.51). The combinations of PDA supplemented with 12 mL L^{-1} of the product tested showed no fungal growth for *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae*, and a slight but not statistically significant growth for *P. ostreatus*, indicating more effective results than all other combinations. Instead, *B. cinerea* appears to be less sensitive to changes in the type of culture medium, with equal performances across PDA and PDA + pine bark biostimulant (at different concentrations). In general, the differences in fungal growth values, based on the different culture media, suggest that the type of medium can significantly influence the development of fungal colonies.

Regarding the evaluation of the percentage of growth inhibition, Nduagu et al.'s formula [28] allowed us to calculate the growth inhibition [%] of the tested fungi compared to the control at the end of the observations.

Regarding the culture medium with 12 mL L^{-1} of the biostimulant, the colonies on which this concentration had the greatest effect, in terms of growth inhibition, were *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae* with total inhibition (100%). They are followed in descending order by *P. ostreatus*, *F. oxysporum* and *A. niger* in which growth inhibitions of 86.21%, 61.20% and 60.37% were found, respectively.

At 6 mL L^{-1} of biostimulant, the reduction was partial and variable, with percentages ranging from 9.93% to 82.99% depending on the fungal species.

At 1.2 mL L^{-1} of biostimulant, only for four species was there an inhibition, from 30.16 to 80.00%, respectively, for *A. niger*, *A. tubingensis*, *F. oxysporum* and *P. ostreatus.* In some cases, mycelial growth was not completely inhibited, showing a stimulation of the colony compared with the control, in particular for *C. gallica* (−0.88%), *F. mediterranea* (−2.07%) and *P. richardsiae* (−6.98%).

In the case of *B. cinerea*, the biostimulant did not cause an inhibition (0%).

4. Discussion

Concerning the concentration of 12 mL L^{-1} of the product tested, the results showed three different behaviors of the fungal colonies: no development and moderate or total development, compared with the control. In particular, the results indicate that PDA with 12 mL L^{-1} of biostimulant was effective in inhibiting fungal growth, showing fungistatic activity for *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae*. In three cases there were similar performances across PDA and PDA with 6 or 12 mL L^{-1} of biostimulant. This could mean that *C. gallica*, *F. mediterranea* and *P. richardsiae* appear to be less sensitive to changes in the type of culture medium and the behavior is the same as growth on PDA, showing a fungistatic activity only at the higher concentration of biostimulant. Instead, *A. niger*, *A. tubingensis*, *F. oxysporum* and *P. ostreatus* showed different activities between PDA and PDA with the pine bark biostimulant at different concentrations. In particular, they showed significantly lower growth on PDA + biostimulant media than the PDA, with marked differences between the different concentrations used also at the lowest concentration. *B. cinerea* behaves equally for the different culture media and no concentration of the biostimulant was able to reduce mycelial growth, even slightly. This is probably due to an enzymatic or metabolic adaptation of the strain to the active compounds present in the biostimulant. This behavior requires further investigation, ideally by employing multiple *B. cinerea* strains isolated from different matrices, in order to better evaluate the strain-dependent sensitivity to the tested compounds. Previous studies have demonstrated both significant differences in the sensitivity of *B. cinerea* strains to plant-derived compounds [29] and a high degree of genetic, enzymatic and pathogenic variability among isolates obtained from different matrices [30].

Regarding the mycelial reduction, these results indicate that the biostimulant has a significant effect on the mycelial growth of the fungal species tested, with higher effectiveness at higher concentrations.

Pinus bark represents a rich source of active compounds with antifungal, antibacterial and antioxidant properties [31]. Bark extract of *Pinus pinaster* Aiton contains several phenolic compounds such as catechins, taxifolin and phenolic acids, and they have received considerable attention because of their antimutagenic, anticarcinogenic and high-antioxidant activities [32]. Within the species belonging to the *Pinus* genus, according to a multitude of studies, the composition of the bark contains a series of ever-present compounds which, between different species, vary only in terms of quantity [33,34]. The compounds most present within the bark of the species belonging to the *Pinus* genus generally consist of polyphenolic compounds, in particular, tannins (which are divided into hydrolyzable tan-

nins and condensed phlobaphenes), lignin, essential oils (terpenes) and polysaccharides (cellulose and hemicellulose) [33,35].

Many studies have tested the antifungal effect of pine bark, and the results are in accordance with each other. Some studies have attested to the effectiveness of pine bark extract in controlling the growth activity of some fungi by conducting *in vitro* tests to evaluate the effectiveness of some conifer bark extracts. An example is the evaluation of pine bark against the wood decay agent *Lenzites trabea* (Pers.) Fr., where it was noted that a certain fungistatic property of the bark of *Pinus resinosa* Aiton, and especially *P. strobus*, effectively contained the growth of the pathogen, probably due to the high presence of tannins [6]. A further recent study conducted by [10] aimed at evaluating the effectiveness of some bark extracts of dicotyledons and conifers, including *P. pinaster*, against two wood decay agents such as *Trametes versicolor* (L.) Lloyd and *Coniophora puteana* (Schumach). P. Karst. also demonstrates in this case high control activity and fungistatic properties of the extract produced from the bark [10]. In another study, the antifungal activity of pine and spruce bark extracts were observed against some strawberry fungal pathogens (*Botrytis cinerea*, *Colletotrichum acutatum* J.H. Simmonds, *Phytophthora cactorum* (Lebert and Cohn), J. Schröt and *Mycosphaerella fragariae* ((TuL) Lindau), and bark extracts showed very high antifungal activity against *B. cinerea* (mycelial growth inhibition 65–100% on the second day of measurements). Obtained data indicated that inhibition decreased during the incubation period of fungi [36]. Instead, in the study which is the subject of this article, the biostimulant did not show any activity against *B. cinerea*, possibly because coniferous trees have an extremely high number of organic compounds of enormous chemical diversity depending on the species [37]. In fact, in another study, bark extracts from four pine species (*Pinus strobus* L., *Pinus douglasiana* Martinez, *Pinus caribaea* Morelet and *Pinus leiophylla*) were evaluated showed a certain level of growth inhibition on the test fungus, *Trametes versicolor* (L.:Fr.) Pilàt, and the bark extract of the species *P. strobus* was slightly more toxic than that of the other species. The antifungal potential probability of tested extracts can be strongly attributed to substances present in bark, such as waxes and pinosylvins, which are the main extracted compounds using hexane and acetone, respectively [38]. In the same way, another study confirmed *in vitro* potential of *Pinus sylvestris* L. bark extracts against Botryosphaeriaceae. It was reported that Scots pine extract exhibited toxic effects on *Heterobasidion annosum* (Fr.) Bref., *Nectria ditissima* Tul. C.Tul. and *Ceratocystis polonica* Siem. (C. Moreau), and moderate [39], levels of pine bark extract toxicity were reported for several other fungi such as *Schizophyllum commune* Fr., *Gloeophyllum trabeum* (Pers.) Murrill and *Fibroporia vaillantii* (DC.) Parmasto [40]. In another case, *Coniophora puteana* (Schumach.: Fr.), P. Karst and *T. versicolor* were tested for a reduction in mycelia growth using a maritime pine extract [10]. All of these studies showed the biocontrol potential of *Pinus* sp. bark extract, in particular its antifungal effect.

Although the precise antifungal mechanism of pine bark extract has not been fully elucidated within the scope of this study, several plausible hypotheses can be proposed based on its complex phytochemical profile, supported by existing literature. Pine bark is particularly rich in phenolic compounds, and various studies on plant-derived antimicrobial agents indicate that flavonoids often inhibit fungal growth through multiple mechanisms. They include disruption of the plasma membrane, induction of mitochondrial dysfunction and inhibition of cell wall formation, cell division and protein synthesis, as well as efflux-mediated pumping systems [41,42].

5. Conclusions

Most of the studies on the activity of commercial formulations indicated as biostimulants or similar are aimed at defining their effects on plant physiology. Less known in the

literature are the interactions with the biotic etiological agents of plant diseases. However, in this experimental work it is still difficult to be able to identify the cause–effect relationships of the components of this biostimulant, based on pine bark extract, in relation to the effects found *in vitro*.

Numerous studies have shown that natural compounds derived from plants are efficient in treating fungal infections. In many cases, essential oils, extracts and active compounds from plants are efficient antifungal agents. Additionally, they are strong and less toxic against phytopathogenic fungus than synthetic fungicides, and are promising alternatives for controlling phytopathogenic fungi. Likewise, they could partially or entirely replace the use of chemical antifungals, which increase resistance in fungi and pollute the environment, generating an eco-friendly control mechanism as well as lowering costs for agricultural companies [38].

The present study showed the potential fungistatic activity of a product made from pine bark extract against plant fungal pathogens. In particular, the investigation conducted, which is to be considered a preliminary study, has highlighted that the application of this tested product can have, at least from what emerged *in vitro*, direct effects on plant pathogens. This biostimulant, based on pine bark extract, had a good efficacy in inhibiting the growth of fungi at the concentration of 12 mL L^{-1}. This effect was particularly marked on *A. tubingensis*, *C. gallica*, *F. mediterranea* and *P. richardsiae* (total inhibition), moderate on *A. niger*, *F. oxysporum* and *P. ostreatus,* with no effect on *B. cinerea*.

These results highlight the potential of the pine bark biostimulant at 12 mL L^{-1} as an effective antifungal agent against several important phytopathogens, suggesting a possible role in integrated disease-management strategies. The observed tolerance in *B. cinerea* may be attributed to intrinsic biological characteristics, which require further investigation.

Although these results are promising, future studies will focus on the mechanisms of action of the molecules contained in the product tested. Ongoing studies are evaluating the metabolomic profile of the product to better characterize the bioactive components responsible for the observed effects.

Further investigations will focus on the evaluation of the effects of the tested product under field conditions. It should be considered that in laboratory experiments (*in vitro*) the pine bark biostimulant was applied directly to fungal colonies, whereas in the field it will need to be tested on plants (both healthy and infected). In particular, in vivo assays are planned in greenhouse conditions on vegetable crops and in open-field conditions on tree crops, testing three concentrations of the biostimulant and the control.

In light of these considerations, the use of the product tested could be indicated for the containment of some phytopathogenic agents, both preventively and with curative effects, if they are absorbed by the same infected host and if they have the same effect that occurs *in vitro*. Biological control is an important component of integrated management of pathogens, such as fungal pathogens, with the aim of reducing the use of chemicals. Future investigations should examine the in vivo activity of this biostimulant, and some tests are ongoing in order to evaluate its potential biocontrol effects on sick plants affected by fungi.

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