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Trichoderma Secondary Metabolites Active on Plants and Fungal Pathogens

Francesco Vinale[1,*], Krishnapillai Sivasithamparam[2], Emilio L. Ghisalberti[3], Sheridan L. Woo[1,4], Marco Nigro[1,4], Roberta Marra[1,4], Nadia Lombardi[1,4], Alberto Pascale[1,4], Michelina Ruocco[1], Stefania Lanzuise[1,4], Gelsomina Manganiello[4] and Matteo Lorito[1,4]

[1]*CNR – Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), Via Università 133, 80055 Portici, Italy*

[2]*School of Earth and Environment – Faculty of Natural and Agricultural Sciences, The University of Western Australia, W.A. 6009, Australia*

[3]*School of Chemistry and Biochemistry – Faculty of Life and Physical Sciences, The University of Western Australia, W.A. 6009, Australia*

[4]*Dipartimento di Agraria, Università degli Studi di Napoli 'Federico II', Via Università 100, 80055, Portici, Italy*

Abstract: Beneficial microbes typically produce bioactive molecules that can affect the interactions of plants with their pathogens. Many secondary metabolites may also have antibiotic properties, which enable the producing microbe to inhibit and/or kill other microorganisms i.e. competing for a nutritional niche. Indeed, some of these compounds have been found to play an important role in the biocontrol of plant diseases by various beneficial microbes used world-wide for crop protection and bio-fertilization. In addition to direct toxic activity against plant pathogens, biocontrol-related metabolites may also increase disease resistance by triggering systemic plant defence activity, and/or enhance root and shoot growth. Fungi belonging to the *Trichoderma* genus are well known producers of secondary metabolites with a direct activity against phytopathogens and compounds that substantially affect the metabolism of the plant. The widescale application of selected metabolites to induce host resistance and/or to promote crop yield may become a reality in the near future and represents a powerful tool for the implementation of IPM strategies.

Keywords: Fungal interactions, plant protection, secondary metabolites, *Trichoderma*.

1. INTRODUCTION

Considerable crop losses incurred by plant diseases caused by phytopathogenic agents. Interest in biological control of fungal pathogens has increased recently in order to find alternatives to the use of chemicals. Synthetic pesticides are costly, pollute the environment and are potentially harmful to animals and humans. Furthermore, their repeated use promotes the development of chemically resistant pathogen strains.

The use of microbes for pest management in agriculture is one of the most effective strategies of biological control. The outcomes of using beneficial microbes are strain dependent and the advantages for the associated plant include: 1) establishment of an antagonistic microbial community in the rhizosphere; 2) suppression of pathogens; 3) overall improvement of plant health; 4) growth promotion; 5) increased nutrient availability and uptake, and 6) enhanced host resistance to both biotic and abiotic stresses [1-3].

*Address correspondence to this author at the CNR – Istituto per la Protezione Sostenibile delle Piante (IPSP-CNR), *Via* Università 133, 80055 Portici, Italy; Tel: +39 081 2539338; Fax: +39 081 2539339;
E-mail: f.vinale@ipp.cnr.it

The modes of action of beneficial microbes include: inhibition or parasitism of pathogens by using antibiotics often in combination with extracellular cell wall-degrading enzymes; competition for nutrients (i.e. iron, nitrogen or carbon) in colonization sites; stimulation of plant resistance mechanisms and development [4, 5].

Although not essential for their primary metabolic processes, microbes, and particularly fungi, produce various secondary metabolites (SMs), including compounds of industrial and economic relevance [6]. SMs are chemically different natural compounds of relatively low molecular weight (in most cases < 3 kDa), that are mainly produced by microorganisms and plants and typically associated to individual genera, species or strains. SMs are biosynthesized from primary metabolites in specialized pathways (i.e. polyketides or mevalonate pathways derived from Acetyl Coenzyme A, or amino acids) and some genes are clustered together. The expression of these genes appears to be induced by one or a few global regulators [6]. SMs show several biological activities possibly related to survival functions of the organism, such as competition against other micro- and macroorganisms, symbiosis, and metal transport.

In fungi, the production of SMs has been often correlated to specific stages of morphological differentiation, and

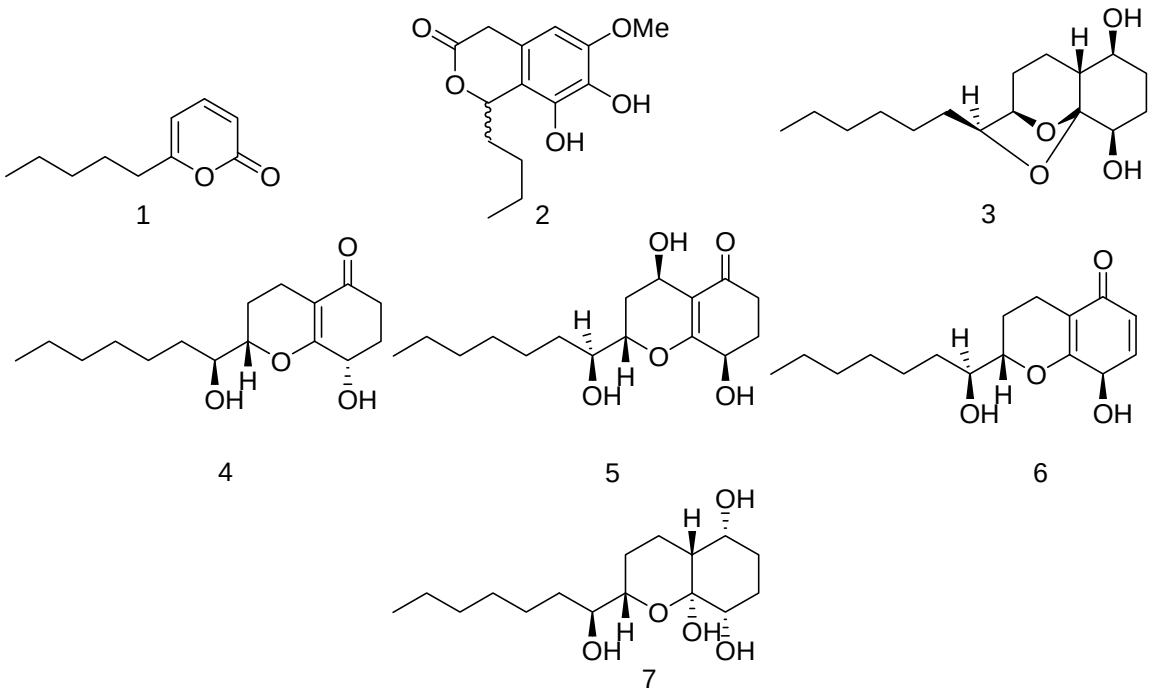

Fig. (1). Chemical structure of: 1-6-pentyl-a-pyrone; 2. cytosporone S; 3,4,5,6,7. Koninginins A, B, D, E and G.

associated to the phase of active growth [7-9]. Their distribution ranges from a limited number of related species to single strains, indicating the absence of a universal function [7-9].

SMs exhibit several biological functions and play an important role in regulating interactions between organisms [10]. Some example are phytotoxins (SMs produced by fungal pathogens that attack plants), mycotoxins (SMs produced by fungi that colonize crops capable of causing disease and death in humans and other animals), pigments (colored compounds also with antioxidant activity) and antibiotics (natural products capable of inhibiting or killing microbial competitors) [7-9]. However, biological activities are not necessarily confined to one specific group or single metabolites [10, 11].

Interestingly, some fungal secondary metabolites can modify the growth and the metabolism of plants, while others seems to target specific fungal processes such as sporulation and hyphal elongation [7]. Microbial metabolites are involved in several biological activities having important consequences in crop production. This review focuses on the main secondary metabolites produced by beneficial fungi and their roles in the complex interactions between the producing microbe and fungal pathogens or plants.

2. SECONDARY METABOLITES THAT ARE TOXIC TO OTHER FUNGI

Fungal SMs have been largely applied in human medicine, providing important drugs such as the antibiotics penicillin and cephalosporins, the immunosuppressant cyclosporine and the antihypercholesterolemic agents lovastatin and compactin [12-17].

The involvement of toxic metabolites in plant disease and particularly in the interactions between beneficial and pathogenic fungi has been conclusively demonstrated by several studies [18]. Antibiotic production by antagonistic fungi is a well-documented phenomenon and often related to the strain biocontrol ability [19, 20].

Biocontrol isolates belonging to *Trichoderma* genus are well known producers of SMs that are toxic for phytopathogenic fungi [21, 22]. The following paragraphs reports the most significant SMs isolated from *Trichoderma* spp. that have shown a significant antifungal activity.

Pyrones

The pyrone 6-pentyl-2H-pyran-2-one (6-pentyl-α-pyrone or 6PP – Fig. **1**) is a metabolite commonly purified in the culture filtrate of different *Trichoderma* species (*T. viride, T. atroviride, T. harzianum, T. koningii*) and is responsible for the coconut aroma released by axenically developed colonies. 6PP has shown both *in vivo* and *in vitro* antifungal activities towards several plant pathogenic fungi and a strong relationship has been found between the biosynthesis of this metabolite and the biocontrol ability of the producing microbe [23, 24].

6PP biosynthesis in *T. atroviride* "is regulated by the G protein Tga1; other uncharacterized metabolites, however, are overproduced in tga1 mutants" [25]. The transcription factor Thctf1 also regulates the biosynthesis of 6PP in *T. harzianum*. Chromatographic and spectroscopic analyses

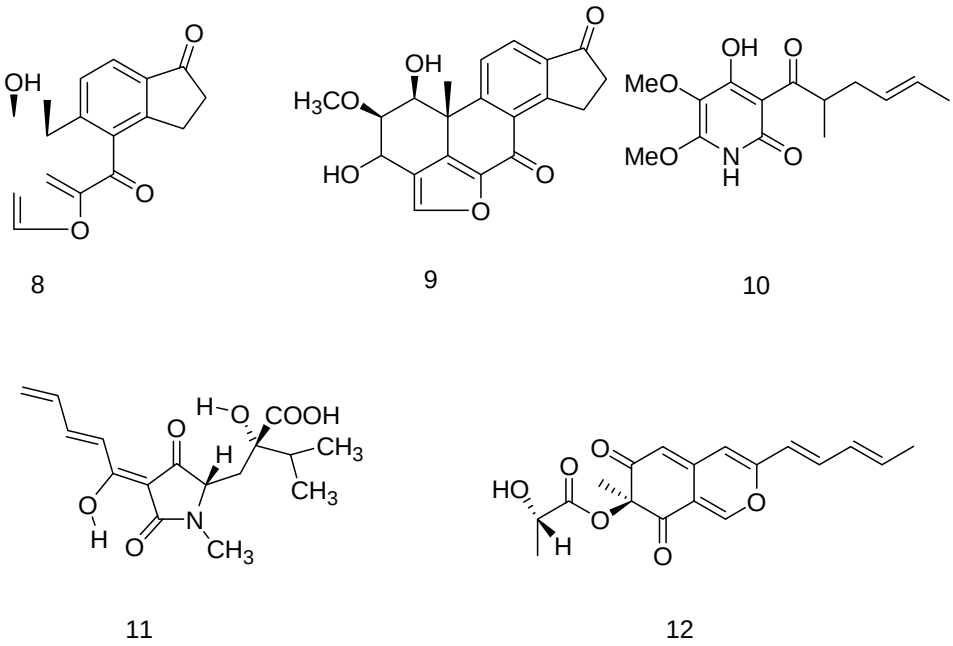

Fig. (2). Chemical structure of: 8 viridin; 9 viridiol; 10 harzianopyridone; 11 harzianic acid; 12 T22azaphilone.

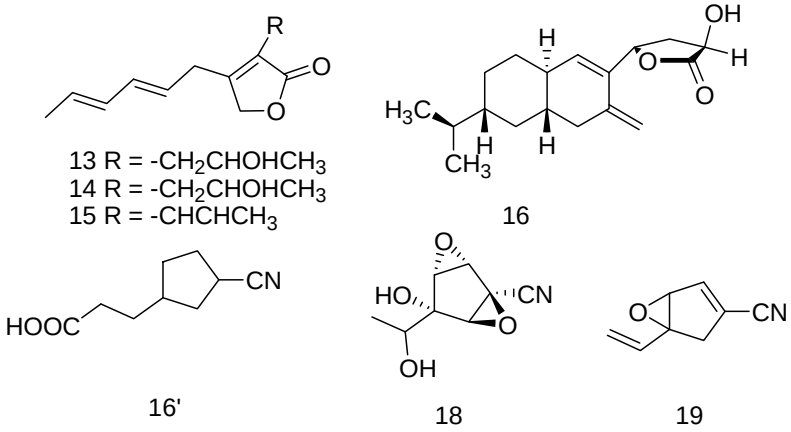

Fig. (3). Chemical structure of: 13 Harzianolide; 14 T39butenolide; 15 deydro-harzianolide; 16 cerinolactone; 16 Dermadin; 18 trichoviridin; 19 homothallins.

showed that Thctf1 null mutants did not produce two secondary metabolites derived from 6PP (6-[(1′R,2′S)-dihydroxypentyl]-2H-pyran-2-one and 6-((1′S,2′R)-2′-propyloxiran-1-yl)-2H-pyran-2-one) and not previously described in the *Trichoderma* genus, that are present in wild-type culture filtrates [26, 27].

Another pyrone named cytosporone S (Figs. **1**-**2**), recently isolated from a *Trichoderma* sp. strain, has been reported to have *in vitro* antibiotic activity against several bacteria and fungi [28].

Koninginins

Koninginins are complex pyranes isolated from *T. harzianum*, *T. koningii* and *T. aureoviride*. Koninginins A, B, D, E and G (Figs. **1**; **3**-**7**) showed *in vitro* antibiotic activity towards the take-all fungus *Gaeumannomyces graminis* var. *tritici* [29, 30]. "Koninginin D (22) also inhibited the growth of other important soil-borne plant pathogens, such as *Rhizoctonia solani*, *Phytophthora cinnamomi*, *Pythium middletonii*, *Fusarium oxysporum* and *Bipolaris sorokiniana*" [31].

Viridins

The steroidal metabolite viridin (Figs. **2**-**8**) is an antifungal compound isolated from diverse *Trichoderma* spp. (*T. koningii*, *T. viride*, *T. virens*) [32, 33]. This molecule prevents spore germination of *Botrytis allii*, *Colletotrichum lini*, *Fusarium caeruleum*, *Penicillium expansum*, *Aspergillus niger* and *Stachybotrys atra* [21, 34].

T. viride, *T. hamatum* and certain *Gliocladium* species produce viridiol (Figs. **2**-**9**), a similar antifungal and phytotoxic metabolite for which the *in vivo* activity has been demonstrated [35, 36].

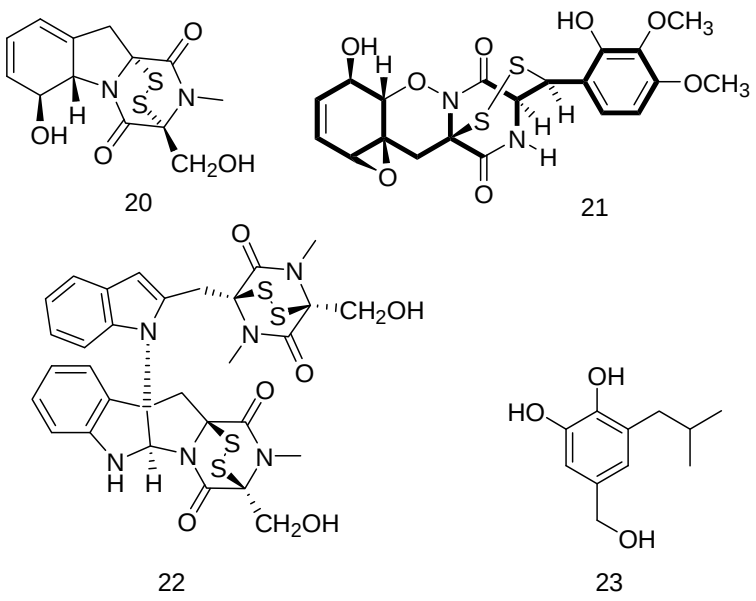

Fig. (4). Chemical structure of: 20 gliotoxin; 21 gliovirin; 22 chaetomin; 23 bigutol.

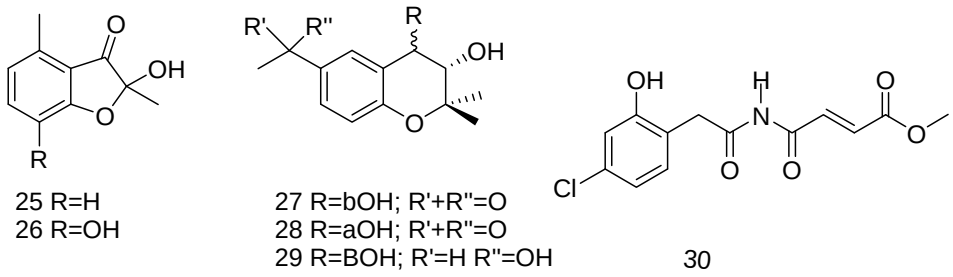

Fig. (5). Chemical structure of: 24 macrosphelide A; 25 3(2H)-benzofuranones A; 26 3(2H)-benzofuranones B; 27 chromanes A; 28 chromanes B; 29 chromanes C; 30 coniothyriomycin.

"A transcriptional comparison of wild type and a viridin and viridiol deficient *T. virens* mutant resulted in the identification of six genes similar to those involved in secondary metabolism of other fungi. Four of these genes (three cytochrome P450s and a cyclase) are located as a cluster that is associated with the production of viridin" [37].

Nitrogen Heterocyclic Compounds

Harzianopyridone (Figs. **2-10**), a *T. harzianum* metabolite containing a penta-substituted pyridine ring system with a 2,3-dimethoxy-4-pyridinol pattern, is a potent antibiotic active against *Botrytis cinerea*, *R. solani* [38], *G. graminis* var. *tritici* and *Pythium ultimum* [39].

Recently, a new compound named harzianic acid and characterized by the presence of a pyrrolidindione ring system (Figs. **2-11**) has been isolated from a *T. harzianum* strain. This tetramic acid derivative showed *in vitro* antibiotic activity against *Pythium irregulare*, *Sclerotinia sclerotiorum* and *R. solani* [40].

Azaphilones

The azaphilones are natural products containing a highly oxygenated bicyclic core and a chiral quaternary center. A new azaphilone, named T22azaphilone (Figs. **2-12**), that showed *in vitro* a marked growth inhibition of several plant pathogens (*R. solani*, *P. ultimum* and *G. graminis* var. *tritici*) has been isolated from liquid culture of *T. harzianum* T22 [39].

Butenolides and Hydroxy-Lactones

Harzianolide (Figs. **3-13**) and its derivatives, T39butenolide (Figs. **3-14**) and deydro-harzianolide (Figs. **3-15**), have been isolated from different strains of *T. harzianum* [29, 39, 41, 42]. The *in vitro* antifungal activities of these SMs was demonstrated against several phytopathogenic agents [29, 39].

A novel hydroxy-lactone derivative, named cerinolactone (Figs. **3-16**), has been isolated from culture filtrates of

T. cerinum. The isolated compound showed *in vitro* antifungal activity against *P. ultimum*, *R. solani* and *B. cinerea* [43].

Isocyano Metabolites

Trichoderma spp. also produce isocyano metabolites that have a characteristic 5-membered ring. However, the isolation and separation of these compounds is very difficult due to their instability [21]. Dermadin (Figs. **3-16'**) from *T. viride* [44, 45], *T. koningii* [46] and *T. hamatum* [47] is an antibiotic metabolite that was patented in 1971 [48]. The isonitrile trichoviridin (Figs. **3-18**) isolated from *T. koningii* [46-49] and *T. viride* showed *in vitro* antibiotic properties [50]. Several dermadin and trichoviridin analogous have also been isolated. Interestingly, *T. koningii* produces various cyclopentenes isocyano metabolites named homothallins (Figs. **3-19**) that affect the morphology of of *Phytophthora* spp. [21].

Diketopiperazines

Gliotoxin (Figs. **4-20**) and gliovirin (Figs. **4-21**) are the two most important *Trichoderma* secondary metabolites belonging to this class of compounds. "P group strains of *Trichoderma* (*Gliocladium*) *virens* produce the antibiotic gliovirin which is active against *P. ultimum,* but not against *R. solani.* Strains of the Q group produce gliotoxin, which is very active against *R. solani,* but less against *P. ultimum"* [51]. In seedling bioassay tests, strains of the P group were able to effectively control *Pythium* damping off on cotton, while Q group strains gave better results towards the same disease caused by *R. solani* [52, 53]. These studies clearly indicate the potential role of antibiotic production in the biocontrol mechanism of the gliotoxin/gliovirin producers.

"The *T. virens veA* ortholog *vel1* (VELVET protein Vel1) is involved in regulation of gliotoxin biosynthesis, biocontrol activity and many other secondary metabolism-related genes" [54, 55]. Moreover, deletion of the 4-phospho-pantetheinyl transferase in *T. virens* resulted in a mutant that failed to induce resistance in *Arabidopsis* and inhibit pathogenic fungi through the production of antibiotics [56].

Peptaibols

Peptaibols are linear peptides rich in non-proteinogenic amino acids (i.e. α-aminoisobutyric acid and isovaline), acetylated at the N-terminal group and the C - terminus is an amino alcohol (i.e. phenylalaninol, valinol, leucinol, isoleucinol or tryptophanol) [57]. Lorito *et al.* [58] "demonstrated that peptaibols inhibited β-glucan synthase activity in the host fungus, while acting synergistically with *T. harzianum* β-glucanases. The inhibition of glucan synthase prevented the reconstruction of the pathogen cell walls, thus facilitating the disruptive action of β-glucanases". The most widely known peptaibol is the *T. viride* alamethicin. The terms peptaibiome and peptaibiomics (peptide antibiotics or peptaibiotics) have been suggested to describe the analysis and study of all peptaibols expressed in an organism or tissue [57] using spectrometric methods, like LC/ESI-MS^n [59] or intact-cell MALDI-TOF [60]. Large multifunctional enzymes known as peptide synthetases assemble peptaibols (non-ribosomal biosynthesis) by the multiple carrier thiotemplate mechanism from a remarkable range of precursors, which can be N-methylated, acylated or reduced [61].

The role of toxic metabolites during the fungus-fungus interaction has been demonstrated also for *Chaetomium globosum*. The ability of several *C. globosum* strains to produce chaetomin (Figs. **4-22**) was correlated with the ability to suppress *Pythium* damping off of sugar beet in pasteurized soil. Activity of chaetomin on *P. ultimum* was 10 times higher than that of the *Trichoderma* diketopiperazine gliotoxin [62].

Two antifungal metabolites (particularly against *R. solani*) named bigutol (Figs. **4-23**) and its derivative methylbigutol, were isolated from three isolates of the mycoparasite *Verticillium biguttatum* [63].

Coniothyrium minitans, a mycoparasite of *Sclerotinia sclerotiorum* and *S. cepivorum* sclerotia, produced four closely related metabolites able to inhibit fungal growth. The main compound, identified as macrosphelide A (Figs. **5-24**), inhibited *in vitro* the mycelial growth of *S. sclerotiorum* and *S. cepivorum* also at very low concentrations [64].

Two 3(2H)-benzofuranones and three chromanes (Figs. **5**; **25-29**) were isolated from another strain of *C. minitans* when the fungus was grown in artificial medium where the only carbon source was sterilized and ground sclerotia of *S. sclerotiorum* [65].

Finally, the coniothyriomycin (Figs. **5-30**) isolated from a *Coniothyrium* sp. showed remarkable fungicidal activities [66].

3. METABOLITES THAT INHIBIT BIOACTIVE MOLECULES MADE BY THE OTHER FUNGI

Regarding secondary metabolites produced by beneficial fungi that inhibit bioactive molecules made by other fungi, only few examples are reported in literature. Examples of "SMs inhibitors" eventually relevant for biocontrol applications include the following compounds that act towards phytopathogenic fungi at specific infection stages. They may be produced by certain antagonistic and endophytic fungi also in pure culture [67]. Flaviolin (Figs. **6-31**) is a non-toxic inhibitor of conidium germination in *Magnaporthe grisea*, that was isolated from liquid cultures of an unidentified Ascomycete [67]. This substance accumulates as an oxidized intermediate product of melanin biosynthetic pathway, especially in the presence of inhibitors of the 1,3,6,8-tetrahydroxynaphthalene reductase such as the commercial fungicide tricyclazole. Similarly, another selective inhibitor of conidium germination in *M. grisea* that showed no effects on vegetative growth is tenuazonic acid (Figs. **6-32**), obtained from cultures of an endophytic *Cladosporium* sp. [67].

Four glisoprenins were purified from cultures of the antagonistic fungi *Clonostachys rosea* as inhibitors of appressorium formation in *M. grisea.* The most active of them was glisoprenin C (Figs. **6-33**) [68, 69].

Appressorium formation in *M. grisea* was also specifically inhibited by the neobulgarones A–F, six dimeric anthraquinone derivatives isolated from mycelium of the

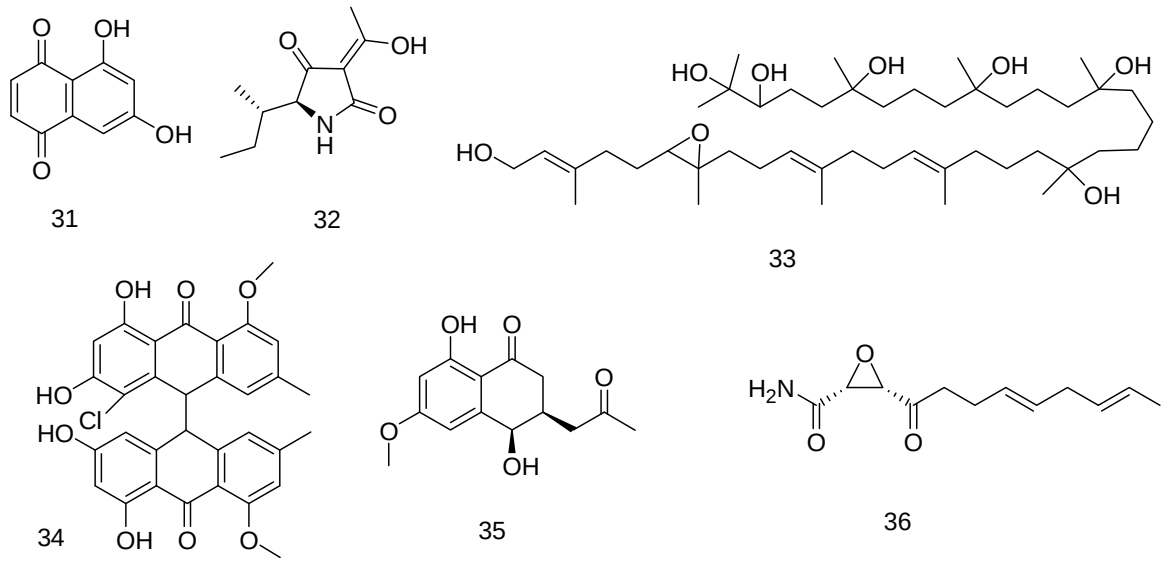

Fig. (6). Chemical structure of: 31 flaviolin; 32 tenuazonic acid; 33 glisoprenin C; 34 neobulgarone D; 35 scytalol D; 36 cerulenin.

ascomycete *Neobulgaria pura* with neobulgarone D, **34** in Fig. (**6**), the most active compound [70].

Several non-toxic natural products, like scytalol D (Fig. **6**-**35**), from the anamorphic fungus *Scytalidium* sp. [71] and cerulenin (Fig. **6**-**36**) from *Cephalosporium caeruleus* [72], inhibited melanin biosynthesis, a very important process for the formation of an effective fungal appressorium [67].

Detoxification of fungal toxins by beneficial microorganisms also represents an interesting possibilities for disease management. Pure cultures of fungi able to detoxify mycotoxins have been obtained from complex microbial populations by using enrichment culture techniques [73]. In particular, *T. viride* and other fungal species were able to degrade aflatoxin B1 [74]. Moreover, *T. harzianum* hydrolases were able to degrade aflatoxin B1 (AFB1) and Ochratoxin A (OTA) *in vitro*. The mycotoxin content in corn flour inoculated with 100 ppb of AFB1 was reduced by up to 30% after treatment with fungal culture filtrates. The enzymatic mixture was separated by gel filtration and fractions with a molecular weight of approximately 100 kDa showed the maximum capacity of mycotoxin degradation [75].

In substrates with high C/N ratios, *T. virens* (formerly *Gliocladium virens*) produced a metabolite similar to the antibiotic viridin (Fig. **2**-**8**), called viridiol (Fig. **2**-**9**), that acts as a plant growth inhibitor [36]. This compound, isolated also from *T. hamatum*, inhibited the 5'-hydroxyaverantin dehydrogenase, an enzyme included in aflatoxin biosynthesis, thus reducing or completely blocking the production of this mycotoxin during the fungal interactions [76, 77].

Elad *et al.* [78] found that mycoparasitism or antibiosis were not the main biocontrol mechanisms of *T. harzianum* strain T39 against *B. cinerea*. These authors indicated that the antagonist interferes with the infection process by affecting the pathogen conidia in the early stages of the interaction. They suggest that T39 acts directly by inhibiting *B. cinerea* hydrolytic enzymes, or indirectly by blocking plant responses that induce enzymatic activity in *B. cinerea*. Subsequently, Elad and Kapat [79] demonstrated that a *Trichoderma* protease is involved in the biocontrol of *B. cinerea* by degrading the hydrolytic enzymes required for infection by the pathogen.

4. METABOLITES THAT SUPPORT COMPETITION FOR NUTRIENTS

Competition for carbon, nitrogen and iron plays an important role during the interactions between beneficial and detrimental fungi, and is associated with the biocontrol mechanisms of non-pathogenic *Fusarium* and *Trichoderma* species [3].

"*Trichoderma* has a strong capacity to mobilize and take up soil nutrients, which makes it more efficient and competitive than many other soil microbes" [3]. This process could be related also to the production of organic acids, such as gluconic, citric and fumaric acids, which decrease soil pH and allow the solubilization of phosphates, micronutrients and mineral cations like iron, manganese and magnesium [3].

Iron is a mineral essential nutrient for numerous microorganisms, both bacteria and fungi [80]. "As a transition metal, redox properties of iron allow it to exist in two oxidation states, ferrous (Fe^{2+}) and ferric (Fe^{3+}) for the donation and acceptance of electrons, respectively" [80]. In the aerobic environment (with oxygen and neutral pH) iron exists mainly as Fe^{3+} and tends to form not soluble hydroxides and oxyhydroxides, making it not available for microbes growth [80].

Microorganisms that excrete siderophores (low-molecular weight; 200-1000 Da) are able to grow in natural environments poor in iron using residual not available iron [81, 82]. Most fungi produce various siderophores, which help the microbes to overcome adverse conditions.

Typically, extracellular and intracellular siderophores are found in fungi that transport or store ferric iron [81].

Secreted metabolites form extracellular Fe(III) complexes. "Then, the iron-charged siderophore is taken up by ferric-chelate-specific transporters or the siderophore-bound Fe(III) undergoes reduction to Fe(II), which is catalyzed by free extracellular or membrane-standing ferric-chelate reductases. If not already released extra cytoplasmatically, the iron has to be removed from the Fe-siderophore complex in the cytosol. This is mediated either by intracellular ferric-siderophore reductases or, in a few cases, by ferric-siderophore hydrolases" [83].

During the steps of iron utilization, the acquired metal is transferred through intracellular trafficking pathways, which may include diverse storage compartments in order to be directed to cofactor assembly systems and to final protein targeting [80].

Transport of siderophores is an energy-dependent process and is stereoselective, depending on recognition of the metal ion coordination geometry. In addition to iron transport, siderophores produced by microorganisms have other functions and effects, including enhancement of pathogenicity, storage of intracellular iron and suppression of microbial growth during the competition with other microbes [80].

The production of microbial siderophores can be beneficial to plants for two reasons: i) siderophores can solubilize iron unavailable for the plant; ii) siderophore production by non-pathogenic microorganisms can also suppress the growth of plant pathogens by depriving them of iron sources [84].

The majority of the fungal siderophores isolated so far belongs to hydroxamate class and can be divided into three structural families: fusarinines, coprogens and ferrichromes [85]. Fungi typically produce more than one siderophore, even if restricted to a particular family. However, a few cases have been reported of fungi able to synthesize siderophores from different structural families [86].

In order to study the importance of iron concentration for the activity of a *T. asperellum* strain (T34), Segarra *et al.* [87] analysed the effect of iron during the interaction of T34 with *Fusarium oxysporum* f.sp. *lycopersici* on tomato plants. In these experiments "Fe competition is one of the key factors for the biocontrol activity of T34 against the pathogen, as an increase in Fe concentration in the nutrient solution lead to the suppression of T34 siderophore synthesis and thus to the inhibition of Fe competition" [87]. Moreover, T34-treated plants significantly enhanced plant growth and development as compared to control plants at high level of Fe (1,000 μM), even though T34 did not reduce the Fe content in leaves or stems. In another work T34 increased the Fe concentration in the aerial parts of lupin plants grown on a calcareous medium. These results clearly indicate a role of siderophores during the interaction with the pathogen and the plants, although no siderophores have been isolated and characterized so far from the culture filtrate of this beneficial microbe [88].

Anke *et al.* [86] isolated siderophores from all different structural families simultaneously from a culture filtrate of *Trichoderma* spp. In particular, the culture filtrate of this fungus obtained in iron deficiency condition contained coprogen, coprogen B, fusarinine C and ferricrocin (Figs. **7**; **37-40**).

In a recent work a new method for the analysis of iron-chelating metabolites using LC-HRMS was used to detect extracellular siderophores produced by 10 different *Trichoderma* strains. *T. harzianum* produced the highest number of siderophores and did not have any unique compounds; while, *T. reesei* biosynthetized one cis-fusarinine as the major siderophore and three others that were present only in *T. harzianum*. The data suggest "that the high diversity of siderophores produced by *Trichoderma* spp. might be the result of further modifications of the non-ribosomal peptide synthetase (NRPS) products and not due to diverse NRPS-encoding genes" [89].

Recently, the ability of the *Trichoderma* SM harzianic acid (Figs. **2**-**11**) to bind with a good affinity essential metals such as Fe^{3+} has been demonstrated [90].

5. METABOLITES INVOLVED IN BENEFICIAL FUNGUS-PLANT INTERACTIONS

SMs that Increase Systemic Resistance

Several metabolites produced by beneficial fungi are involved in the induction of plant resistance, such as: i) "proteins with enzymatic activity, i.e. xylanase" [91]; ii) "avirulence-like gene products able to induce defence reactions in plants" [2]; and iii) "low-molecular-weight compounds released from either fungal or plant cell walls" by specific enzyme activities [2, 92, 93]. "Some of the low-molecular-weight degradation products released from fungal cell walls have been purified and characterized, and found to consist of short oligosaccharides comprised of two types of monomers, with and without an amino acid residue" [92, 93]. "These compounds elicited a reaction in the plant when applied to leaves or when injected into root or leaf tissues. Further, they also stimulated the biocontrol ability of fungi such as *Trichoderma* by activating the mycoparasitic gene expression cascade" [92].

Some *Trichoderma* SMs may act as elicitors of plant defence mechanisms against pathogens. A reduction of disease symptoms on tomato and canola seedlings treated in particular with 6PP (Fig. **1**) and inoculated, respectively, with the pathogens *B. cinerea* or *Leptosphaeria maculans* has been reported [94]. Moreover, "soil drench applications of 6PP four days before inoculation with *Fusarium moniliforme* showed considerable suppression of seedling blight and substantial plant growth promotion, compared with the untreated control" [95]. Application of 6PP on maize seedlings distinctly enhanced the activities of peroxidase, polyphenoloxidase and β-1,3-glucanase in both shoot and root tissues indicating an induction of defence responses in maize plants [95].

Peptaibols are another class of plant defence elicitors produced by *Trichoderma*. Application of alamethicin, a long sequence peptaibol with a 20-residue produced by *T. viride*, induced defence responses in *Phaseolus lunatus* (lima bean) [96] and *Arabidopsis thaliana* [97]. Moreover,

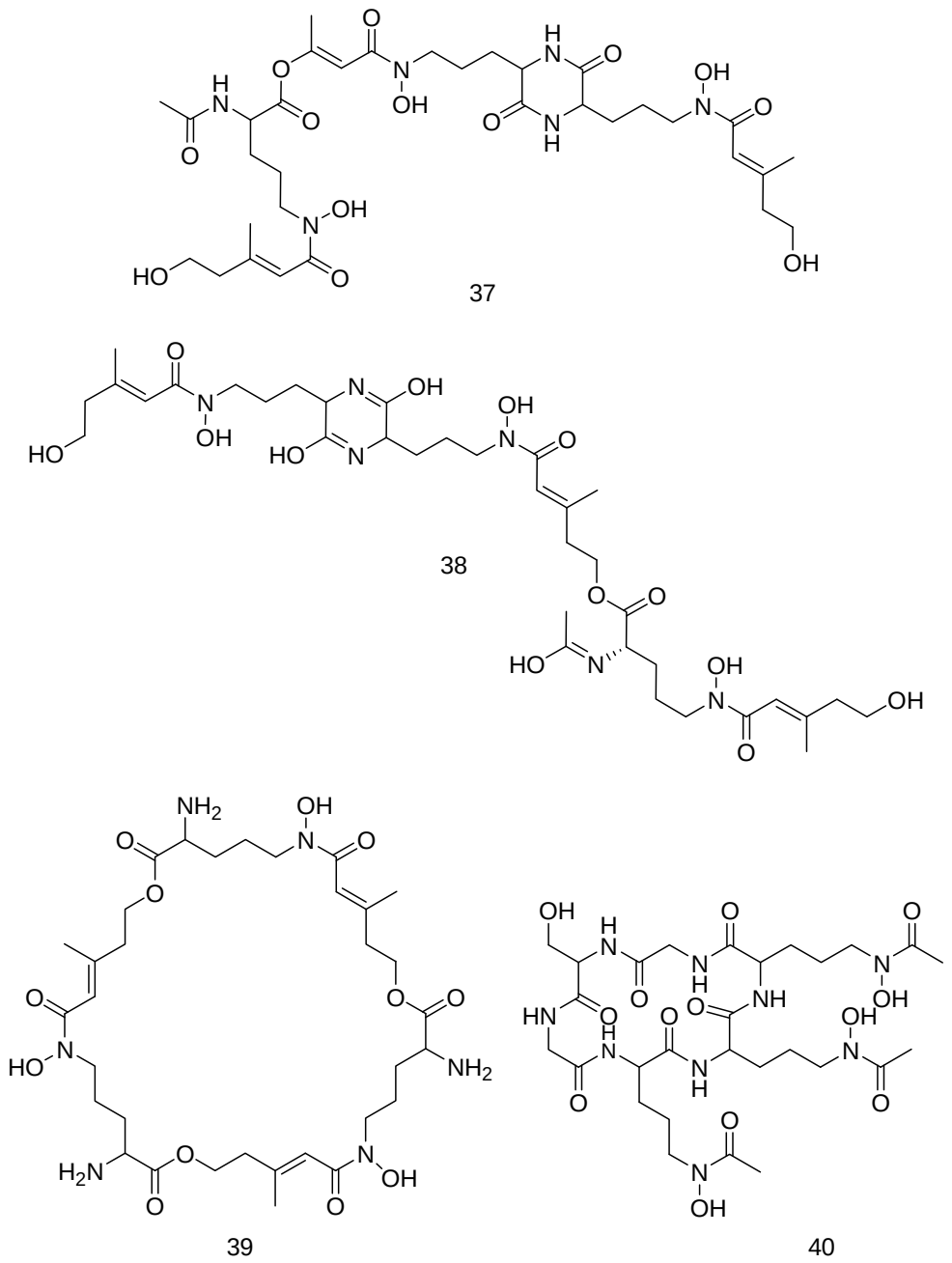

Fig. (7). Chemical structure of: 37 coprogen; 38 coprogen B: 39 fusarinine C; 40 ferricrocin.

disruption of the non-ribosomal peptide synthetases (NRPS) gene, *tex1*, resulted in the loss of production of all forms of 18-residue peptaibols in *T. virens* which corresponded to a significant reduction of the ability of the fungus to induce systemic resistance responses [98].

Recently Mukherjee *et al.* [99] demonstrated that mutation in one of the polyketide synthase/non-ribosomal peptide synthetases "(PKS/NRPS) hybrid genes reduces the ability of *T. virens* to induce the defence response gene pal (phenylalanine ammonia lyase), suggesting a putative role for the associated metabolite" [99] (derived from polyketide pathway) product in induced systemic resistance. These results provide evidence that a PKS/NRPS hybrid enzyme responsible for the metabolite production is involved in *Trichoderma*–plant interactions resulting in induction of defence response in maize.

SMs that Enhance Growth, Development and Yield

Many beneficial fungi including some *Trichoderma* species, can increase plant growth, development and yield also in the absence of pathogens [100-104].

Several strains belonging to the *Trichoderma* genus, including some used world-wide in agriculture, have been found to be able to stimulate plant development, especially at the root level (i.e. formation of more lateral roots) by activating an auxin-dependent mechanism [105] and/or producing indole-3-acetic acid (IAA) or auxin analogues [106].

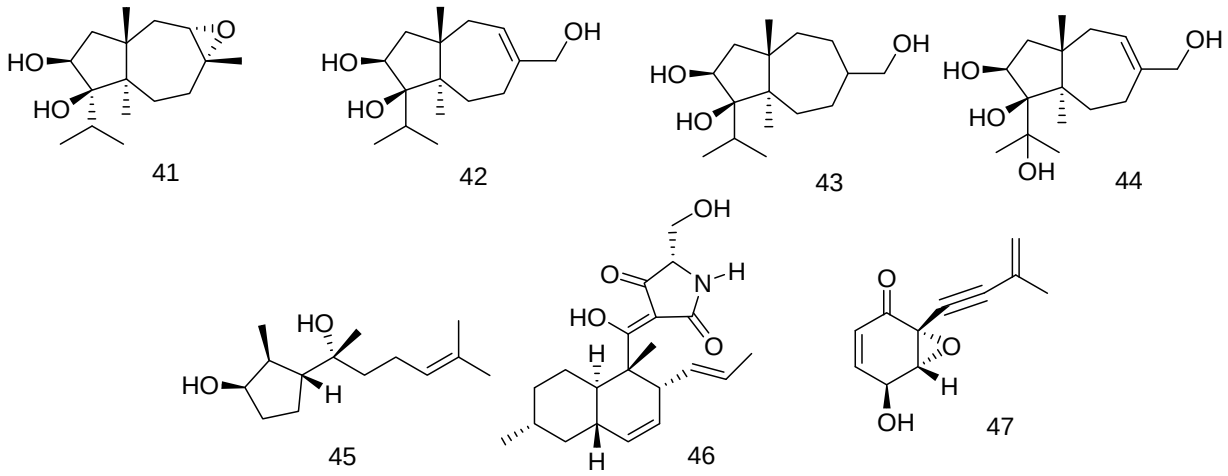

Fig. (8). Chemical structure of: 41, 42, 43, 44 trichocaranes A – D; 45 cyclonerodiol; 46 trichosetin; 47 5-hydroxy-1-(3-methyl-3-buten-1-ynyl)-7-oxabicyclo[4.1.0]-hept-3-en-2-one.

Positive effect on plant development have been demonstrated for several *Trichoderma* SMs [107]. Koninginins (Fig. **1**; **3**-**7**), 6PP (Fig. **1**), trichocaranes A – D (Fig. **8**; **41**-**44**), harzianopyridone (Fig. **2**-**9**), cyclonerodiol (Fig. **8**-**45**), harzianolide (Fig. **3**-**13**) and harzianic acid (Fig. **2**-**11**) are examples of isolated compounds that affect plant growth in a concentration dependent manner [30, 108-114].

A dual culture fungus/plant provides a simple method of establishing their interaction and allows the isolation of metabolites induced by one of the two components. Interestingly, dual culture of *T. harzianum* and calli of *Catharathus roseus* produced an antimicrobial metabolite called trichosetin (Fig. **8**-**46**), that was absent in single cultures. This compound was probably produced by the fungus [115]. Trichosetin affected negatively growth and development of numerous plant species [116] and is a *N*-desmethyl analogue of equisetin, a tetramic acid isolated from *Fusarium equiseti* [117].

A novel metabolite, named cerinolactone (Fig. **3**-**16**), has been isolated and characterized *T. cerinum* and was able to positively alter the growth of tomato seedlings three days after treatment [43].

A sterile dark ectotrophic fungus isolated from roots of *Neurachne alopecuroidea* produced 5-hydroxy-1-(3-methyl-3-buten-1-ynyl)-7-oxabicyclo[4.1.0]-hept-3-en-2-one in liquid cultures (Fig. **8**-**47**). The metabolite showed antimicrobial activity against some plant pathogens and improve plant growth and development, similarly to what found for the ectotrophic fungus applied *in vivo* [118].

The dose-effect response of plant growth to secondary metabolites produced by beneficial fungi clearly deserves further investigation. These metabolites may possibly act as auxin-like molecules, which have a positive effect at low concentrations while having an inhibitory effect at higher doses. For instance, an hormone activity was detected on etiolated pea stems treated with harzianolide and 6PP. These compounds also affected the growth of tomato and canola seedlings in a manner depending on the concentration and/or the application method used [94].

CONCLUSION

Hundreds of SMs produced by beneficial fungi have been isolated and characterized. Here we focused only on the compounds produced by fungal microorganisms that have been involved in the interactions with phytopathogenic agents and/or plants having a positive outcome for agriculture.

In terms of a sheer number, fungal SMs with a direct antibiotic activity against plant pathogens have been mainly isolated from biocontrol strains of the genus *Trichoderma*. Even though many SMs are known, elite strains usually produce only a few main SMs. The quality and the quantity of SMs synthesized depend on: i) the compound considered; ii) the species and the strain; iii) the occurrence of other microorganisms; iv) the equilibrium among elicited biosynthesis and biotransformation rate; v) the growth conditions. Further, in some cases, the biocontrol agent was able to modulate the production of toxic SMs according to the presence or the absence of the target pathogen [119].

Interestingly, SMs by beneficial fungi may also be involved in biocontrol mechanims through the inhibition of bioactive products produced by fungal pathogens. Examples of metabolites that inhibit other fungal products or bioactive molecules include compounds able to detoxify or inhibit the biosynthesis of mycotoxins, which is very harmful for humans and animals. Further studies on this interesting topic may produce some new biotechnological tools to effectively reduce contamination and losses of human food and animal feed.

It is well recognized that biocontrol fungi, such as selected agents of *Trichoderma* spp., are able to produce

compounds with multiple activities, including direct/indirect toxic effects against plant pathogens, plant defence induction or growth promotion [3]. Several fungal species, as well as numerous chemically different substances, have been found to be able to modify plant development and crop yield. An hormone-like effect has been proposed for some *Trichoderma* SMs and specific antimicrobial compounds having this characteristics have been detected in plant-fungus cultures. In fact, treatment with *Trichoderma* metabolites produces extensive changes of the plant expressome, proteome and metabolome, by acting on specific pathways involved in the synthesis of major hormones, resistance to biotic/abiotic stresses and nutrient uptake [107]. These recent findings have suggested new strategies for the development of novel bioformulates based on microbial metabolites alone or in combination with live microbes, in order to maximize the beneficial effects and reduce the risks associated with the release of microrganisms into the environment.

The current techniques, such as metabolomics and expressomics, could provide novel information about the molecular factors involved in the complex interactions occurring between plants, beneficial microbes and phytopathogenic agents. Nevertheless, it is important to continue with the identification of natural compounds that are not produced under usual laboratory conditions [120]. Therefore, novel techniques developed recently need to be used in order to select *Trichoderma* strains for biocontrol that are able to produce only or mainly "beneficial" metabolites (i.e. antibiotics not toxic to plants and their consumers and/or acting as plant growth promoters and/or inducers of disease resistance) [120].

The application of selected metabolites to induce host resistance and/or to promote crop yield may be an interesting alternative to chemicals. Regardless, further studies aimed at conclusively determining the nature and the fate of mixtures of SMs released in the soil or the phyllosphere by beneficial fungi applied as biocontrol or fertilization agents by employing "inundative methods", are still needed.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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