

Proceedings

Trichoderma: fungi able to meet the needs of the eco-friendly agriculture and nutraceutical industry

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Abstract:

Trichoderma strains are hyphomycetes used as biological disease control and as growth promoters of the plants. *Trichoderma* strains and some of their metabolites promote the activation of secondary metabolites involved in plant defense mechanisms of action, including the production of phenolic compounds. The latter have antioxidant, anti-inflammatory, and antimicrobial properties, making them helpful in preparing functional foods, supplements, and nutraceutical and nutricosmetic products. This work summarizes the potential of *Trichoderma* in olive trees cultivation, the effects on the environment, the potential on the production of phenolic compounds, the analytical procedures used to identify and dose secondary metabolites, and the opportunities for the pharmaceutical, nutraceutical, and cosmetic industries.

Keywords: phenols; nutraceutical; *Trichoderma*; food waste recovery.

1. Introduction

The constantly growing world population (it is thought that 9.1 billion people will populate the Earth in 2050) has increased the demand for food [1]. Some chemical fungicides are used in agriculture for disease control since fungal pathogens are the most limiting factor of crop production [2]. Unfortunately, some fungicides and derivatives are harmful to humans and the ecosystem [3–4]. Therefore biological control of pathogens seemed a helpful strategy to solve this problem. The biological control employs microorganisms to decrease the pathogenics' growth by damaging their metabolisms and/or creating a parasitic relationship [5] in a dose-dependent manner [6]. Some *Trichoderma* strains are used for this purpose [7] since they are symbiotic with plants, invasive, avirulent, opportunistic, able to enhance plant growth and decrease the pathogen's (fungi, nematodes, and bacteria) growth [8–9]. *Trichoderma* fungi can directly interact with pathogens (e.g., competing for nutrient and space, hyperparasitism, and as antibiosis) [10] or enhance the plant's defense system. Plant roots release chemical signals which attract *Trichoderma*. The improvement of the hydrophobins (cysteine-rich proteins) production facilitates the

anchor of the expansin-like proteins [11], the root invasion [12], and the decreased levels of the phytoalexin allow rapid colonization of root tissues [13]. In this study, the potential of *Trichoderma* in olive trees cultivation, its ability to improve the phenolic compounds' production, the analytical procedures used to identify and dose secondary metabolites, and the opportunities for the industries (e.g., pharmaceutical, nutraceutical, and cosmetic) are revised.

2. Olive tree

The olive tree (*Olea europaea* L.) is the only species of the Oleaceae family that produces edible fruits [14]. It is widely cultivated in Mediterranean countries and several subtropical regions of Australia, South America, and Southern Africa. Olive trees have a high tolerance to poor soils, salinity, drought [15], and excess of boron [14]. The soil fertility and the volume of irrigation water affect their productivity [16]. Low soil fertility, drought, and high temperatures enhance the tendency of olive trees for alternate bearing, with higher yields being produced every second year [17] due to a decrease in mineral uptake [18]. Some pathogenic fungi (e.g., *Verticillium dahliae*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Pythium* sp) infect the root making rot and wilt diseases. The wilting disease caused by *Verticillium dahliae* is the most severe disease of olive trees worldwide [19]. Some agrochemical fungicides are employed to combat them. Unfortunately, some of these harm humans and the environment. Therefore it was thought to improve the health and production of the olive tree by using beneficial microorganisms [20].

3. *Trichoderma* strains in olive tree cultivation

Trichoderma harzianum makes hydrolytic enzymes and antibiotics (regulated by ethylene-dependent signaling pathway and jasmonic acid) that activate plant defense responses [21]. *Trichoderma harzianum* Ths97 strain acts against *Fusarium solani* strain Fso14 responsible for the root rot in olive plants [21]. *Trichoderma asperellum* Bt3 and T25 strains are active against *Verticillium wilt* [22]. *Trichoderma* strains (T22 and KV906) and their metabolite 6-pentyl- α -pyrone (6PP) promote leaf number and branch length growth [23]. The solarization, improve the *Trichoderma* performance against *Verticillium* [24].

4. Environmental effect of olive tree cultivation with *Trichoderma* strains and its metabolites

The *Trichoderma* strains play an essential role in the bioremediation of soil contaminated with pesticides. They can decrease the polycyclic aromatic hydrocarbons (e.g., endosulfan pentachlorophenol, dichlorodiphenyltrichloroethane) [25], phenols, nitrates, cyanides [26], and Cr (VI) [27-28] from the soil. ATP-binding cassette (ABC) transporter protein systems are involved in resistance against insecticides (e.g., organochlorines, carbonates, and organophosphates) [29]. Moreover, *Trichoderma* strains and their metabolites increase the phenolic levels in waste products from olive tree cultivation (e.g., leaves) and EVOO processing (e.g., pomace, and vegetation water) as well as in parts of the plant intended for human consumption, transforming these waste products into

important biomolecules' sources for the pharmaceutical, food and cosmetic industries. Noteworthy is the possibility to employ *Trichoderma*'s secondary metabolites in olive tree cultivation since they do not have the limitations of living biological control agents, including storage and dosage [30].

5. Effect of *Trichoderma* strains on phenolic compound production in olive tree cultivation

Phenolic compounds have an aromatic ring with one or more hydroxyl substituents. Plants produce them in response to biotic and abiotic factors. They help the plant with antioxidant and antimicrobial properties and humans with their anti-inflammatory, antioxidant, antitumor, anti-obesity, and cardioprotective activity [31]. Olive trees produce, above all, secoiridoids, phenolic alcohols, lignans, flavonoids, and phenolic acids in variable concentrations based on the part of the plant (e.g., leaves, fruit, roots) and environmental stimuli [30]. *Trichoderma* strains M10 and TH1, and some of their metabolites (6PP and harzianic acid, HA) improve phenolic concentrations in leaves, as well as *Trichoderma* strains (T22 and GV41) and metabolites (6PP and harzianic acid) in extra virgin olive oil (EVOO), pomace and vegetation waters [32] probably enhancing the plant nutrient uptake mechanism [33] and the efficient use of nitrogen [34–35]. The higher flavonoids' concentration in the leaves than EVOO can be explained since the flavonoids are produced in the leaves [32]. The biostimulation with T22 mainly improves the secoiridoids' concentration, flavonoids' and lignans' levels in the EVOOs showing a diverse action of the biocontrol agents on the Shikimate pathway [30]. The significant improvement in polyphenols' level following the biocontrol treatments with fungi than their metabolites depends on fungi' ability to attach the pathogen with more mechanisms (e.g., antibiosis, competition, direct mycoparasitism, and disease resistance's induction) simultaneously and synergistically [36]. The different phenolic profile in EVOO, pomace, and vegetation water is due to their different solubility [32, 37]. Dini and coll. have validated a method to dosage the phenols in EVOO, which consists of isolating phenols by solid-phase extraction) and dosing them by Folin-Ciocalteu method. The latter is a spectrophotometric method based on the reaction between Folin–Ciocalteu reagent and the hydroxy groups of phenolics and absorbance reading at λ 765 nm [38–39]. Other methods (not always validated) involve the use of innovative technologies for extraction (e.g., ultrasound-assisted or microwave-assisted extractions) combined with chromatographic techniques (HPLC, UPLC) for purification [40].

6. Effect of *Trichoderma* strains on VOC compounds production in olive tree cultivation

Plant volatile organic compounds (VOCs) work as protective compounds [41–43]. They produce metabolomic, transcriptomic, and behavioral responses in herbivores and insect pollinators. The VOCs' composition and concentration influences plant-plant interactions and plant-insect [44]. It is known that olive trees (*Olea europaea* L.) produce VOCs, including C6 compounds, hydrocarbons, C9 aldehydes, and sesquiterpenes,

depending on the growth time and environmental conditions (e.g., the water status) [45]. *Trichoderma* strains (TH1, T22, M10, KV906, and GV41,) and their secondary metabolites (HA, and 6PP) increase VOCs production, disturbing three biosynthetic pathways: lipid-signaling, methylerythritol 1-phosphate (MEP), and shikimate pathways. Strains (KV906, GV41) and the metabolite HA regulate the lipid-signaling pathway and enhance the hydrocarbon aldehyde formation. Strains (GV41, T22, M10, TH1), and the metabolites (HA and 6PP) act on the Shikimate pathway and increase aromatic compounds syntheses. Strain M10 and the metabolites (6PP and HA) act on MEP (methylerythritol 1-phosphate) and improve monoterpene synthesis.

7. Conclusions

Some *Trichoderma* strains and metabolites have shown a high potential in the cultivation of olive trees (*Olea europaea*) both for their fungicidal abilities and because they improve the phenolics' production increasing the nutritional interest of the drupes and oil and the economic interest for the recycling of agricultural production waste (e.g., leaves) and oil (e.g., pomace and vegetation water) as sources of bioactive molecules of interest for the pharmaceutical, food and cosmetic industries. Further research on how the *Trichoderma* strains and metabolites act as biocontrol agents against pathogenic fungi is necessary to improve the knowledge of these versatile and polyvalent biocontrol agents.

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