

Natural compounds: an effective and eco-friendly strategies for controlling and combating plant pathogens

Alin DIN^{1,2*}, Ion MITREA¹, Rodi MITREA¹

¹University of Craiova, Faculty of Horticulture, 13 A.I. Cuza Street, Craiova, Dolj, Romania; din.alin96@yahoo.com (*corresponding author); mitreaion@yahoo.com; rodimitrea@yahoo.com

²National Research and Development Institute for Biotechnology in Horticulture Stefanesti Arges, Romania

Abstract

Currently, the management of phytopathogenic agents is an ongoing challenge globally, as they cause major damage in agricultural plantations. However, the use of synthetic pesticides currently in use has negative effects on the environment and on the living organisms that develop their life cycle in these habitats. For this reason, research in the last decade has led to the identification and exploitation of natural compounds with important antifungal properties as natural alternatives to combat and control pathogens. This review is based on the description of plant extracts, essential oils and natural compounds with important anti-fungal properties. However, their direct application to plants is difficult, because their adhesion and persistence in environmental conditions does not allow this fact. The current challenge is to develop formulations based on natural compounds that retain their properties over time in order to be applied to agricultural crops.

Keywords: essential oils; natural compounds; plant protection; phytopathogens; plant extract

Introduction

Phytopathogenic fungi represents infectious agents of plants, producing changes in different stages of plant development, even after harvesting during storage. There are numerous fungal species that cause serious damage to agricultural products, because they affect the quality of fruits and vegetables (nutritional values, organoleptic characteristics, etc.). More than 25% of cereals (wheat, corn, rice, etc.) are contaminated in most cases by fungi belonging to the genera *Fusarium* and *Aspergillus*, which are indirectly responsible for the production of aflatoxin B1, which is toxic and highly carcinogenic, as well as more than 300 fungal metabolites, which can generate metabolic disorders or even poisoning. (Satish *et al.*, 2007; Díaz-Dellavalle *et al.*, 2011; Lee *et al.*, 2007). In the study by Stracquadanio *et al.* (2021), it was shown that natural extracts obtained from *Trichoderma asperellum* and *Trichoderma atroviride* applied to tomatoes, wheat and maize, reduced the production of ochratoxin A. In addition, the extracts reduced the production of mycotoxins in a dose-dependent manner and with a long-lasting effect.

Plants are a rich source of different chemical compounds such as: alkaloids, flavones and flavonoids, phenols, terpenes, tannins or quinones. Produced as secondary metabolites, they can constitute up to 30% of the dry mass of plants, having an essential role in their protection against pathogens, abiotic stress factors, but

Received: 08 Nov 2022. Received in revised form: 20 Mar 2023. Accepted: 22 Mar 2023. Published online: 29 Mar 2023.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

also against herbivores. Due to their specific properties, plants have been used since ancient times for medicinal purposes, but also as food additives. Nowadays, the recognition of the chemical structure and functions of certain plant compounds allows us to extract and isolate them for use as ingredients in the cosmetic, food, pharmaceutical, industries etc. There is also great interest in their application as biopesticides, fungicides and insecticides to protect crop plants (Mazid *et al.*, 2011; Bhagat *et al.*, 2014).

The use of phytopreparations but also of essential oils represents the true means of perspective for the biological protection of plants, having numerous advantages, such as: the use of these preparations has demonstrated an effective impact on phytopathogens, the extraction is not complicated and long-lasting, does not pose a danger to the environment and people, they decompose quickly in agroecosystem, also proving an increased efficiency (Zarins *et al.*, 2009). Studying of the effectiveness of plant extracts led to the premise that they can be used as alternative bioformulations to the currently used synthetic fungicides, due to their antifungal activities, the low level of toxicity for humans and the environment, but also biodegradation. (Du Plooy *et al.*, 2009; Tripathi and Dubey, 2004).

Plant extracts and essential oils

Algae extracts have been shown to be a possible substitute for synthetic pesticides, as demonstrated by the study of Jiménez *et al.* (2011) who evaluated the antifungal potential of extracts from the brown alga *Lessonia trabeculata* and observed that it reduced tomato leaf lesions following *Botrytis cinerea* infection. They also observed that aqueous and ethanolic extracts of the red alga *Gracillaria chilensis* inhibited the growth of *Phytophthora cinnamomi*. In addition, there are numerous studies that refer to the antifungal activity of brown algae extracts against fungal species, *Cladosporium herbarum*, *Botrytis cinerea*, *Cladosporium herbarum*, *Phialophora cinerescens*, *Phoma tracheiphila*, *Sclerotinia sclerotiorum*, *Sclerotium rolfsii* and *Verticillium dahliae* being just a few species of fungi pathogens that showed sensitivity to algae extracts obtained from species belonging to the *Phaeophyceae* class (Fenical and Sims, 1973; Moreau *et al.*, 1988; Belattmania *et al.*, 2016; Esserti *et al.*, 2017; Mohamed and Saber, 2019; Vicente *et al.*, 2021). So, it was found that the potential of the activities presented by the metabolites produced by seaweed is strongly influenced by both the environment and the biological factors (Khaleafa *et al.*, 1975; Ballantine *et al.*, 1987; Robles-Centeno *et al.*, 1996; Kumar *et al.*, 2000; Jiménez *et al.*, 2011; Vicente *et al.*, 2021). Ashwani *et al.* (2011) observed high efficacy of aqueous extracts of *Cannabis sativa*, *Parthenium hysterophorus*, *Urtica dioica*, *Polystichum squarrosus* and *Adiantum venustum* against five phytopathogens of high economic importance namely *Alternaria solani* on tomatoes, *Alternaria zinnia* on *Zinnia* sp., *Curvularia lunata* on corn, *Rhizoctonia solani* on potatoes and *Fusarium oxysporum* on tomatoes. Notable results were also obtained by Al-Rahmah *et al.* (2013) who evaluated the antifungal potential of natural extracts obtained from *Lantana camara*, *Salvadora persica*, *Thymus vulgaris*, *Zingiber officinale* and *Ziziphus spina* on phytopathogens found in tomatoes: *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*. The extracts of *Salvadora persica*, *Thymus vulgaris* and *Zingiber officinale* showed a good fungistatic action on the previously mentioned phytopathogenic fungi.

The antifungal properties of the extracts obtained from *Ricinus communis* and *Chromolaena odorata* were tested by Nahunnaro and Bayaso (2012) against the fungus *Alternaria solani*, the results obtained being encouraging in the biological control of this phytopathogen. Thyme essential oil is also known for its antifungal effects, which is why Wu *et al.* (2011) tested the potential against the phytopathogen *Alternaria alternata*, the inhibition percentage being over 60% at the concentration of 500 µL/L. Askarne *et al.* (2012) evaluated the antifungal activity of 50 plant species collected in different regions of southern Morocco. Thus, it was demonstrated that *Anvillea radiata* and *Thymus leptobotrys* completely inhibited the mycelial growth of the pathogenic fungus *Penicillium italicum* at concentrations of 10%. Promising results were also obtained by Şesan

et al. (2015) who evaluated the antifungal potential of natural extracts obtained from *Achillea millefolium*, *Allium sativum*, *Artemisia dracunculus* 'Sativa', *Hyssopus officinalis*, *Mentha* sp., *Rosmarinus officinalis*, *Satureja hortensis*, *Tagetes patula*, *Valeriana officinalis*. The extracts were used in three different concentrations namely 5%, 10% and 20%. The highest efficacy (100%) against the phytopathogen *Botrytis cinerea* was demonstrated by the extract obtained from *Hyssopus officinalis*, at all three concentrations used, followed by the extract from *Satureja hortensis*. The extract obtained from *Allium sativum* and *Mentha* sp. it is observed that they are effective only at high concentration, 10% and 20% respectively. However, the study by Shalini *et al.* (2019) suggest that neem oil and aqueous extracts obtained from *Allium sativum*, *Leucas martinicensis* and *Zingiber officinale* can be used to control the phytopathogen *Diplocarpon rosae*. However, the neem oil tested on the affected plants showed a phytotoxic effect compared to the other extracts used. The inhibitory effect of *Salvia officinalis* extract was tested against the fungus *Phakopsora pachyrhizi* that produces Asian rust, one of the most important diseases of soybean crops. The fungus is very aggressive causing significant damage to farmers, chemical control is not exactly easy. From the studies carried out, a potential substitute for classic pesticides can be considered the extract from *Salvia officinalis* that was studied by Borges *et al.* (2013), they observed a germination percentage below 15%. In 2010, Schuster *et al.* (2010) used extract obtained from the species *Glycyrrhiza glabra* against six plant pathogens, *Phytophthora infestans*, *Pseudoperonospora cubensis*, *Podosphaera xanthii*, *Blumeria graminis*, *Uromyces appendiculatus* and *Botrytis cinerea*. The previous study shows that three of the six phytopathogens, *Phytophthora infestans*, *Pseudoperonospora cubensis* and *Uromyces appendiculatus*, showed increased sensitivity using the 5% concentration of the extract.

Keeping pace with the activity of antifungal extracts, a number of five extracts obtained from *Artemisia absinthium*, *Rumex obtusifolius*, *Taraxacum officinale*, *Plantago lanceolata* and *Malva sylvestris* were tested as natural alternatives in the control and combat of phytopathogenic fungi *Alternaria alternata*, *Penicillium expansum* and *Mucor piriformis*. The percentage of inhibition for all tested extracts being over 60% on the three tested phytopathogens (Parveen *et al.*, 2014). Moreover, *Capsicum* extract shows important antifungal properties against the pathogenic fungus *Colletotrichum gloeosporioides*. (Mihaescu and Neagu Frăsin, 2020).

The efficacy of garlic (*Allium sativum*) extract against the phytopathogen *Fulvia fulva* was reported by Ting-Ting *et al.* (2011) where it was demonstrated that at the concentration of 80 mg/mL⁻¹ the percentage of inhibition is 100%. According to the study conducted by Ramaiah and Garampalli (2015), the extracts obtained from the species *Solanum indicum*, *Azadirachta indica* and *Oxalis latifolia* demonstrated increased efficacy against the phytopathogen *Fusarium oxysporum* f. sp. *lycopersici*, their inhibition percentage being over 70% of the colony diameter.

The essential oils of *Rosmarinus officinalis* and *Thymbra spicata* were tested against the phytopathogen *Monilinia fructigena* and the potential for use of both essential oils was shown to be high. However, *Thymbra spicata* essential oil at a concentration of 2 µl and demonstrated 100% efficacy, while *Rosmarinus officinalis* essential oil at a concentration of 16 µl was shown to completely inhibit mycelial development (Yilar and Bayar, 2018).

In addition, *Cymbopogon citratus* oil at a concentration of 500 ppm has been shown to completely inhibit sporulation and germ tube generation of the fungal species *Colletotrichum coccodes*, *Botrytis cinerea*, *Rhizopus stolonifer* and *Cladosporium herbarum*. (Trösken *et al.*, 2004; Jiménez-Reyes *et al.*, 2019). In contrast, according to the study by Chang *et al.* (2008) the oil of *Calocedrus macrolepisa* var. *formosana* showed an important antifungal activity, up to 65% against *Pestalotiopsis funerea* and 52% against the phytopathogen *Fusarium solani*. Antifungal activity of *Xanthium strumarium* essential oil was demonstrated by Sharifi-Rad *et al.* (2015), where it was found to reduce mycelial growth of the phytopathogenic species *Aspergillus niger* by up to 34%. Another oil with properties to prevent and fight against the pathogen *Botrytis cinerea* is ginger. (Tripathi *et al.*, 2008; Jiménez-Reyes *et al.*, 2019).

Major groups of natural compounds with antifungal potential

Phenolic compounds

In recent years a number of studies have been reported on the antifungal potential of phenolic compounds obtained from natural sources. The positions and number of hydroxyl groups in the phenolic group are believed to be closely related to their toxicity relationship against microorganisms. Thus, the mechanisms considered to be responsible for the toxicity of phenols against microorganisms include enzymatic inhibition by oxidized compounds, possibly by reaction with sulfhydryl groups or by nonspecific interactions with proteins. Among the polyphenols, flavan-3-ols, flavonols and tannins have received the most attention due to their broad spectrum and greater antimicrobial activity compared to other polyphenols, as well as the fact that most of them are able to suppress a number of microbial virulence factors (such as: inhibition of biofilm formation, reduction of host ligand adhesion and neutralization of bacterial toxins) showing synergy with antibiotics (Daglia, 2012). Antibacterial properties of phenolics may also be due to iron deficiency or hydrogen bonding with vital proteins such as microbial enzymes (Sanhueza *et al.*, 2014; Kabir *et al.*, 2015).

Flavones are phenolic structures whose chemical structure contains a carbonyl and a 3-hydroxyl group. Thus, the amentoflavones obtained from *Selaginella tamariscina* demonstrated an important antifungal action against the phytopathogen *Aspergillus flavus* (40 µg/100 µl), a fact demonstrated by Jun Jung *et al.* (2006). A series of four compounds, eupomatenoid-3, eupomatenoid-5, conocarpan and orientin, from *Piper solmsianum* showed antifungal activity against all tested dermatophytes (*Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus niger*, *Microsporium cani*, *Microsporium gypseum*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum*, *Cryptococcus neoformans*, *Candida albicans*, *Candida tropicalis*) with values in the range of 2.0-60.0 mg/ml (De Campos *et al.*, 2005).

The mechanism by which phenolic compounds act focuses on altering the cytoplasmic membrane, causing cell lysis, and can directly inhibit cellular proteins. These mechanisms are what allow the inhibition of microorganisms (Abad *et al.*, 2007; Martínez, 2012). For example, a large number of polyphenols extracted from the leaves of *Rhus muelleri* inhibit the growth of the fungus *Fusarium oxysporum* having a diverse phylogenetic complex (Jasso De Rodríguez *et al.*, 2015). Mendoza *et al.* (2011) demonstrated that the flavonoid 5,7-dihydroxy-3,8-dimethoxyflavone, extracted from *Pseudognaphalium robustum*, reduced mycelial growth and partial spore germination of the fungus *Botrytis cinerea*.

Stilbenes, especially resveratrol and its derivatives, have become famous for their positive effects on a wide range of medical conditions, as indicated by a large number of published studies. A less investigated area of research is their antimicrobial properties. Thus, the research of Caruso *et al.* (2011) performed on the phytopathogenic fungus *Botrytis cinerea* highlighted both the antifungal effect of resveratrol and its potential to inhibit the development of conidia. The demonstrated optimal concentration, according to Adrian *et al.* (1997) being between 60-140 µg/mL. Also, against dermatophytes such as *Trichophyton mentagrophytes*, *Trichophyton tonsurans*, *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporium gypseum*, the inhibitory activity of resveratrol has been shown to be between values of 25-50 µg/mL (Chan, 2002).

Terpenoid compounds

Terpenes are a large class of aromatic chemicals present in a wide variety of plants, foods and essential oils, and their purpose is to protect plants against fungi, bacteria and animal pests. In general, essential oils have a high concentration of terpenes and sesquiterpenes (Abad *et al.*, 2007; Martínez, 2012). The antifungal activity of carrot oil was tested against the pathogen *Alternaria alternata*, the inhibition percentage was 65% of the colony diameter. The main esters found in carrot essential oil are sesquiterpenes such as carotol (Abad *et al.*, 2007). Canelo (*Drimys winteri*) essential oil contains varying amounts of polygodial and drimenol (Muñoz-

Concha *et al.*, 2007). These terpenes have important antifungal properties against the fungus *Gaeumannomyces graminis* (Monsálvez *et al.*, 2010).

Thyme is well known for its antifungal effects, thanks to the thymol found in its oil. Shcherbakova L. *et al.* (2021) through their studies showed that thymol has a remarkable antifungal action. Also, Zhang *et al.* (2016) reported the antifungal efficiency of pure monoterpenes such as: β -citronellol, carvacrol, citral, eugenol, geraniol and thymol against wood white rot fungi: *Trametes hirsuta*, *Schizophyllum commune* and *Pycnoporus sanguineus*. Notable results were also obtained by Xie *et al.* (2017) who confirmed antifungal properties of essential oils from *Origanum vulgare*, *Cymbopogon citratus*, *Thymus vulgaris*, *Pelargonium graveolens*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* against the wood-decomposing fungi *Trametes hirsuta* and *Laetiporus sulphureus* where the following compounds were highlighted: carvacrol, citron, citronellol, cinnamic aldehyde, eugenol and thymol. The studies carried out by Sempere-Ferre *et al.* (2021) on two pathogens *Botryotinia fuckeliana* and *Rhizoctonia solani* highlighted the antifungal effects of some natural compounds (eugenol, carvacrol, thymol and cinnamic aldehyde).

The first report regarding the production of volatile antibiotics was reported by Strobel *et al.* (2007) who succeeded in isolating the compounds: 2-methylbutanoic acid, 3-methylbutanoic acid, 2-methyl-2-butenal, 3-methyl-3-butene-1-OL, guaicol, 1-octene-3-ethyl, formamide, N-(1-methylpropyl), derivatives of azulene and naphthalene, caryophyllene, phenylethyl alcohol and propanoic acid, from *Muscodor albus* species (*Xylariaceae* family), an endophyte of *Guazuma ulmifolia* species (a *Sterculiaceae* plant collected from the tropical forest-western Ecuador). These compounds were tested against some pathogens of high economic importance, namely: *Botrytis cinerea*, *Mycosphaerella fijiensis*, *Pythium ultimum*, *Phytophthora cinnamomi*, demonstrating their effectiveness by inhibiting the development of the mycelium of the fungi. Also, subsequent reports showed that *Muscodor albus* was also found on other host plant types such as *Myristica fragrans*, *Terminalia prostrata*, *Cinnamomum zeylanicum* and *Ginkgo biloba* (Worapong *et al.*, 2001; Ezra and Strobel, 2003; Atmosukarto *et al.*, 2005; Lacey and Naven, 2006; Strobel *et al.*, 2007; Banerjee *et al.*, 2010; Alpha *et al.*, 2015). Diterpenoids, sclareol and 13-epi-sclareol isolated from the resinous extract of *Pseudognaphalium cheiranthifolium*, can inhibit the mycelial growth of the phytopathogenic fungus *Botrytis cinerea*, being able to be used as a natural control strategy (Mendoza *et al.*, 2015).

Alkaloid compounds

Alkaloids are chemical substances with a wide range of biological activities, usually present in species of the *Solanaceae* family. Thus, hyoscyamine isolated from the medicinal plant *Hyoscyamus muticus* has been shown to inhibit the growth of several species, including *Fusarium dimerum*, *Fusarium nivale* and *Fusarium oxysporum*. (Abdel *et al.*, 2009). The study conducted by Soumya and Bindu (2012) highlighted the antifungal potential of capsaicin isolated from the species *Capsicum frutescens*, the tests being performed on four species of fungi, namely *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* sp. and *Rhizopus* sp. Regarding the effectiveness of capsaicin, it was also studied by Buitimea-Cantúa *et al.* (2018) on the phytopathogen *Aspergillus parasiticus*, the inhibitory percentage being set at 50%. The research of Slusarenko *et al.* (2008) demonstrated the effectiveness of allicin obtained from garlic extract against the fungus *Alternaria* sp. Similar results were obtained by Mihaescu *et al.* (2021) who showed that allicin in vitro exhibits a remarkable fungicidal effect against the fungus *Alternaria solani*. Singh *et al.* (2007) demonstrated that alosecurin or phyllocrysin isolated from the root of *Phyllanthus amarus*, completely inhibits the germination of spores of phytopathogenic fungi *Curvularia* sp., *Curvularia lunata*, *Collectotrichum* sp., *Colletotrichum musae* and *Heterosporium* sp.

Conclusions

The need of the modern era is to obtain pathogen-free and high-productivity crops to meet global food requirements. Because of this, the use of plant extracts has emerged as a natural alternative to synthetic fungicides for controlling and combating phytopathogenic fungi. However, research for the identification and characterization of new compounds with antifungal potential is still a challenge, being the main current approaches in the discovery and development of new biopesticides. Also, following the bibliographic study, we could observe that natural extracts and essential oils from plants present active compounds with important effective antifungal properties. Finally, it could be observed that the phenolic structures represent real starting points for the development of new biopesticides due to the effectiveness they have demonstrated. Another class of compounds that have demonstrated their effectiveness is that of terpenoids, of which the best known are: carvacrol, citral, eugenol, geraniol and thymol, specialized studies confirming their antifungal potential.

Authors' Contributions

Conceptualization: AD and RM; Writing - original draft, review and editing: AD, IM and RM; Supervision and validation: AD, IM and RM.

All authors read and approved the final manuscript

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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