

THE USE OF PLANTS TO PROTECT PLANTS AND FOOD AGAINST FUNGAL PATHOGENS: A REVIEW

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Abstract

Background: Plant fungal pathogens play a crucial role in the profitability, quality and quantity of plant production. These phytopathogens are persistent in avoiding plant defences causing diseases and quality losses around the world that amount to billions of US dollars annually. To control the scourge of plant fungal diseases, farmers have used fungicides to manage the damage of plant pathogenic fungi. Drawbacks such as development of resistance and environmental toxicity associated with these chemicals have motivated researchers and cultivators to investigate other possibilities.

Materials and Methods: Several databases were accessed to determine work done on protecting plants against plant fungal pathogens with plant extracts using search terms “plant fungal pathogen”, “plant extracts” and “phytopathogens”. Proposals are made on the best extractants and bioassay techniques to be used.

Results: In addition to chemical fungicides, biological agents have been used to deal with plant fungal diseases. There are many examples where plant extracts or plant derived compounds have been used as commercial deterrents of fungi on a large scale in agricultural and horticultural setups. One advantage of this approach is that plant extracts usually contain more than one antifungal compound. Consequently the development of resistance of pathogens may be lower if the different compounds affect a different metabolic process. Plants cultivated using plants extracts may also be marketed as organically produced. Many papers have been published on effective antimicrobial compounds present in plant extracts focusing on applications in human health. More research is required to develop suitable, sustainable, effective, cheaper botanical products that can be used to help overcome the scourge of plant fungal diseases.

Conclusions: Scientists who have worked only on using plants to control human and animal fungal pathogens should consider the advantages of focusing on plant fungal pathogens. This approach could not only potentially increase food security for rural farmers, lead to commercial rewards, but it is also much easier to test the efficacy in greenhouse or field experiments. Even if extracts are toxic it may still be useful in the floriculture industry.

Keywords: infection, plant extracts, antifungal, phytopathogens

Abbreviations used: PAMP – Pathogen associated molecular patterns, PTI – PAMP-triggered immunity inducible defence, ETI – effector-triggered immunity defence, BTH – Benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester, MIC – minimum inhibitory concentration

Introduction

Plant fungal pathogens cause most of the diseases occurring in agricultural and horticultural setups (Agrios, 2009). Collectively, the phytopathogens have developed mechanisms and ways to attack any plant (Knogge, 1996), seeking entry and sourcing nutrients forcefully for growth and development (Horbach et al., 2011). These pathogens can reproduce asexually and/or sexually (Gould, 2009), and can overcome plant immune defences (Thomma et al., 2011; Zvereva and Pooggin, 2012). This negatively affects the plant health, plant homeostasis, plant physiology and in some cases causes systemic damage (Agrios, 2005).

For plant fungal diseases to occur the plant fungal pathogen must be able to germinate on the surface of a suitable host. The plant fungal pathogen spores can only germinate when conditions are favourable. This includes suitable humidity by rain or dew, availability of low molecular mass nutrients and a suitable host (Osharov and May, 2001). Fungal spores can remain viable for many years using self-inhibitors to stop germination until favourable conditions are present (Chitarra et al., 2004). When the conditions are favourable the plant fungal pathogens form infection structures like the appressorium and the infection peg, for the hyphae to penetrate the host (Schafer, 1994). Pathogens like *Colletotrichum gloeosporioides* that cause diseases in avocados, can use their host waxes to infect their host (Podila et al., 1993).

Plant fungal pathogens can use different strategies to attack and enter their host. Some pathogens enter their host using mechanical pressure and chemical action while others enter their host through wounds and the stomata (Knogge, 1998). Through evolution, plants have developed defences against fungal pathogens. This is a good motivation for investigating the presence of antifungal compounds in plants (Eloff and McGaw, 2014). The plant fungal pathogens need strategies to circumvent plant host defence mechanisms i.e. pathogen associated molecular patterns (PAMP), PAMP-triggered immunity (PTI) inducible defence and effector triggered immunity (ETI) defence (Thomma et al., 2011; Zvereva and Pooggin, 2012). Successful pathogens that can overcome plant PTI and ETI, especially those which have evolved their *avirulent* genes to overcome plant host *R*-resistance genes (Stergiopoulos and de Wit, 2009), cause devastating plant diseases that can lead to epidemics (Dean et al., 2012). Not all plant fungal pathogens are able to cause diseases in the same host. Some have a narrow host range while others have a broad host range. Some plants can be a host to several plant fungal pathogens (Agrios, 2005). Plant fungal pathogens can infect new regions where they have never been present through wind, birds, humans, insects, water and infected parts of plants (Agrios, 2005; Rossman, 2009).

Not all fungal species attack plants but plant fungal pathogens attack every group of plants (Knogge, 1996). These fungi collectively are responsible for 80% of plant diseases (El Hussein et al., 2014). About 8000 fungal species cause nearly 100 000 diseases in plants (Agrios, 2005).

In general, plant fungal pathogens can be grouped into biotrophs, necrotrophs and hemibiotrophs based on the mechanism of infection. Biotrophs are organisms that survive on living tissues causing infections without killing the host. Some of the pathogens use the appressorium to penetrate the host and feeding structure like the haustoria to source nutrients from the surrounding cells. The biotrophs have a limited host range e.g. powdery mildew fungi and rust fungi (De Silva *et al.*, 2016). Necrotrophs infect the living host eventually killing the infected area. This is because necrotrophic fungi can only complete their life cycles on dead tissues. These pathogens continually produce hydrolytic enzymes and toxins to destroy the plant cells. Necrotrophs produce two types of toxins, host specific toxins, that are specific to the plant host and allow the pathogen to cause diseases on a specific host e.g. *Cochliobolus carbonum*. Secondly, broad spectrum toxins enable some of the pathogens e.g. *Sclerotinia sclerotiorum*, *Alternaria brassicicola* and *Botrytis cinerea* to infect and destroy unrelated plant species (Wen, 2013). The hemi-biotrophs use similar mechanisms as biotrophs to cause infections and later kill their host as necrotrophs e.g. *Collettrichium* (Agrios, 2005).

Problems caused by plant fungal pathogens in agricultural production and food spoilage

Agricultural production can deliver sustainable plant products that can alleviate poverty and starvation (Alexandratos and Bruinsma, 2012). Epidemics like late blight diseases of potatoes, cereal rusts and smuts, ergot of rye and wheat, brown spot of rice, coffee rust, Sigatoka disease of banana, chestnut blight, the downy and powdery mildews of grape; wheat stem rust and rubber leaf blight have caused severe damages to agricultural production. Plant fungal pathogens can also pose a threat to human health by decreased plant quality and quantity. This may lead to forced displacement of food, shift economic prospects of countries, cause political uncertainty and forced migration of humans (Anderson et al., 2004; Ellis et al., 2008; Gould, 2009; Singh et al., 2012). These phytopathogens consequently cause enormous problems for farmers, policy makers, researchers and consumers (Fletcher et al., 2006).

Plant fungal pathogens provide a complexity of problems for farmers in plant production as listed in a review of the top ten plant fungal pathogens by Dean et al. (2012). Plant fungal pathogens like *Magnaporthe oryzae* and *Collettrichium* spp. cause destructive diseases worldwide. *Fusarium graminearum* diminishes crop quality and *Blumeria graminis* reduces crop quantity. In a single field, one organism like *Mycosphaerella graminicola* can evolve and infect various plant varieties, reducing the ability of plants to overcome the pathogen infection. Similarly, *F. oxysporum* has over 70 *formae speciales*, making it difficult to identify the relevant pathogen. One species can cause diseases in a variety of plant species, e.g. *Botrytis cinerea* has 200 plant hosts and *F. oxysporum* about 100 plant hosts. A single pathogen like *M. oryzae* can lead to a large loss of grain production. When pathogens coexist with other pathogens like *F. graminearum* with other *Fusarium* species, they can completely shut down plant immune defences. Moreover, pathogens like *M. graminicola* can cause symptomless colonization, for more than 7 days. This makes it difficult to determine if the plant is infected. Some pathogens like *Ustilago maydis* can complete the life cycle within two weeks making them highly destructive. Pathogens like *Puccinia* spp. cause repeated crop failures, this making it difficult to even grow other crops in crop rotation.

Some pathogens can infect new plant species, cause diseases in related plant species or affect multiple plant species. Many weak pathogens in their hosts can cause havoc and epidemics in related species across continents (Burdon and Thrall, 2009). Plant fungal diseases lead to annual economic losses that exceed 200 billion US dollars (Horbach et al., 2011) in pre- and post-harvest processes (Gonzalez-Fernandez et al., 2010). In 1993 alone, the reappearance of wheat and barley scab (causal agent *F. graminearum*) in North America resulted in yield and quality losses estimated at 1 billion US dollars (Mullins and Kang, 2001).

It is clear that fungal pathogens cause many and varied problems with a potential enormous impact on plant production.

Food spoilage and post-harvest problems caused by plant fungal pathogens

Post-harvesting diseases and food spoilage caused by plant fungal pathogens can occur during various stages of processing such as harvesting, handling, storage, packaging and transportation, in hands of the consumer (Agrios, 2005). Fungal pathogens are the main causal agent of fresh fruit and vegetable rot in postharvest processes (Gatto et al., 2011). More than 100 species of fungi are responsible for the majority of postharvest diseases (Tripathi and Dubey, 2004) and postharvest diseases can destroy 10 – 30% of crop yield (Agrios, 2005). In developing countries and tropical regions loss of perishable commodities can be as high as 50% (Tripathi and Dubey, 2004).

Different plant fungal pathogens cause significant losses during storage due to fungal spoilage. Fungi such as *Botrytis cinerea* (fruits - raspberries, strawberries, grapes, kiwi fruit, pears, peaches, plums and cherries; vegetables - carrots, lettuce, peas and beans), *B. allii* (onions and related crops such as garlic), *Penicillium italicum* and *P. digitatum* (green rot of citrus), *Penicillium expansum* (blue rot of apples and pears), *Penicillium glabrum* (onion) and *Penicillium funiculosum* (onion) (Moss, 2008). Fungal species such as *Fusarium*, *Geotrichum* and *Aspergillus* are some the agents of decay of fruit and vegetables causing significant economic losses and undesirable characteristics of the plant commodities (Agrios, 2005). *Colletotrichum* diseases can destroy up to 100% of stored fruits (Dean et al., 2012).

Problems caused by mycotoxins

Mycotoxins are low molecular weight compounds which are produced by moulds. These secondary fungal metabolites are toxic to vertebrates at very low concentrations and play no role in the development and growth of the fungus (Hussein and Brasel, 2001). The mycotoxins are widespread in food and are important because the compounds can cause human and animal diseases such as carcinogenic, teratogenic, tremorogenic, haemorrhagic and dermatitis (Agrios, 2005; Kumar et al., 2008). Furthermore, consumption of contaminated plant foods or animal feed with mycotoxins, can lead to a number of metabolic problems such as liver function deterioration, protein synthesis interference or other disorders such as skin sensitivity, necrosis, or extreme immunodeficiency (Sweeney and Dobson, 1998). Annually, up to 25% of the world crops are contaminated with mycotoxins and this has a severe impact on food security and the economy with around 1 billion tons of food and food products lost each year (Matny, 2015). Mycotoxin related diseases are frequently caused by *Aspergillus*, *Fusarium* and *Penicillium* species (Agrios, 2005). Toxins such as aflatoxins, ochratoxins, trichothecenes, zearalenone, fumonisins, tremorogenic toxins and ergot alkaloids have adverse effects on human and animal health as well as a negative agro-economic impact (Hussein and Brasel, 2001).

Plant fungal pathogens therefore have a huge negative role in the efforts of providing food for the ever-growing population of the world. Plant fungal diseases and the toxins they produce severely threaten agricultural production, by affecting plant commodities quality and quantity leading to pre and post harvesting economic losses. In an effort to minimize damage caused by fungi in agricultural setups various management/control innovations are used.

Control of plant pathogens

Without control or induced plant defences many people in the world will be starving and will suffer from some of the dreadful epidemics and outbreaks occurring from time to time. To overcome persistent attack of plant fungal pathogens on agricultural products, various agrochemical products have been developed and used. Some of these agrochemicals are toxic to humans and a withdrawal period is required between the last dosage and the harvesting. Many of these fungicides also have negative environmental effects on soil organisms and insects and plant pollinators. The use of natural product is getting more attention from farmers in the production of organic food strongly supported by environmentalists and some consumers.

Inducing resistance against fungi

Natural resistance is based on using plant defence molecules in agricultural production to induce resistance against invading fungal pathogens (Nega, 2014). Salicylic acid and its analogues are used to induce systematic acquired resistance in various crops that are struggling with diseases. Researchers have shown that 30 g of benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) can protect the wheat crop against both *Puccinia recondita* and *Septoria* spp. for an entire season (Reignault and Walters, 2007). Jasmonic acid and its derivatives can induce resistance in struggling crops and production of compounds that has health benefits (Wasternack, 2014). Methyl jasmonates suppress postharvest infections of strawberries by *B. cinerea* and reduces decay of 'Marsh Seedless' grapefruit by *P. digitatum* in agricultural setups (Tripathi and Dubey, 2004). Researchers have identified other natural products (chitosan, β -aminobutyric acid, glucosinolates, propolis, fusapyrone, deoxyfusapyrone, ethephon, microbial products, and plants extracts) that induce resistance against fungal pathogens. These products are used around the world to enhance quality and yields in agriculture setups (Tripathi and Dubey, 2004; Thakur and Sohal, 2013).

Fungicides

Since the first use of fungicides in the 1800s, synthetic chemicals have provided much needed relief in the management of plant fungal disease in agricultural production. The introduction of various synthetic chemicals in agricultural productions over the years has reduced the impact of many plant fungal diseases and increased plant crop yield and led to financial gains (Gianessi and Reigner, 2006). Since the 1970s, however farmers have been struggling with the emergence of resistance against fungicides (Ishii, 2006; Possiede et al., 2009). This leads to yield financial losses.

In 1996, the world-wide sales of fungicides amounted to about 5.9 billion US dollars (Martinez, 2012). Annually, the USA spends over 600 million US dollars on synthetic chemicals (Gonzalez-Fernandez et al., 2010). In 2002, Japan had the biggest market of 818 million US dollars in the world for fungicides (Ishii, 2006). Fungicides are frequently toxic to non-target organisms like earthworms, microbes and humans (genotoxicity) causing imbalances in the ecosystems (Nega, 2014, Patel et al., 2014). Many of these chemicals are degraded slowly and are difficult to remove. These may also lead to contamination of water systems and rivers (Stamatis et al., 2010).

Biological control

With all the problems associated with synthetic chemicals, many scientists are investigating biological pesticide solutions (Martinez, 2012; Nega, 2014). Biological pesticides include chemicals derived from microorganisms, plants and animal sources. In USA, there are currently more than 245 registered biopesticide-active ingredients used in hundreds of products. These account for 20% of all pesticides-active ingredients registered in the country (Yoon et al., 2013).

The potential use of microorganisms in the treatment of plant fungal diseases is based on the antagonistic nature of microbes towards the fungal pathogens. The results of experimental and field trials studies of microbial antagonistics against plant fungal pathogens are promising (Sharma et al., 2009). Several fungal and bacterial antagonistic commercial products including products like GiloGard (*Gliocladium virens* – seedling diseases of ornamentals and bedding plants, F-Stop (*Trichoderma harzianum* – several soilborne diseases) BINAB T (*T. harzianum/T. polysporum* – to control wood decay), Gallex or Galltrol (*Agrobacterium radiobacter* K-84 – crown rot) Dagger G (*Pseudomonas fluorescens* – *Rhizoctonia* and *Pythium* damping-off of cotton and Kodiak (*Bacillus subtilis* – seed diseases) are effectively and successfully used worldwide to remedy problems associated with plant fungal diseases (Agrios, 2005).

Using plant extracts

There has been a large numbers of papers published on the *in vitro* antifungal activity of plant extracts. Unfortunately many authors have used methods such as agar diffusion assays that do not work well with plant extracts, mainly because many antifungal compounds in plant extracts are relatively non-polar and these non-polar compounds do not diffuse well in the aqueous agar matrix. It is also very difficult to compare results between different laboratories because many factors influence the agar diffusion results. Eloff and McGaw (2006) and McGaw and Eloff (2010) have discussed different methods that can be used to protect humans or animals against fungi.

A serial dilution method using tetrazolium violet as an indicator of growth to determine antibacterial activity of plant extracts was developed and has been used widely (Eloff, 1998a). This method has been expanded and works very well for fungi (Masoko et al., 2005). With this method, the minimum inhibitory concentration (MIC) of the extracts can be determined. Several authors have recommended that only MICs of 0.1 mg/ml and lower should be considered to have significant activity (Eloff, 2004; Rios and Recio, 2005; Cos et al., 2006). Bioautography also worked very well to indicate the number of antifungal compounds in plant extracts (Masoko and Eloff, 2005).

The best extract in the majority of cases where many extractants were used, was acetone (Eloff, 1988b). In most cases water extracts had very low antimicrobial activity (Kotze and Eloff, 2002). Because traditional healers mainly have water available as an extractant, traditional leads may not be very useful. This led to the random screening of acetone tree leaf extracts against nosocomial bacteria and fungi. When the antimicrobial activity of 717 crude extracts of 537 tree species were determined against four bacteria and two fungi only a few extracts had an MIC higher than 2,5 mg/ml and many extracts had MICs of 0.02 mg/ml and lower (Pauw and Eloff, 2014).

Many plant species have been investigated for their antifungal activities (Eloff and McGaw, 2014; Raut and Karuppaiyil, 2014). Many essential oils inhibit post-harvest fungal infections and prolong shelf-life of many crops in storage conditions (Tripathi and Dubey, 2004). Essential oils also inhibit mycotoxin production of a number of fungal species (Sivakumar and Bautista-Banos, 2014).

Some plants can protect themselves against various phytopathogens (Martinez, 2012). These plants produce a variety of antimicrobials (e.g. phytoalexins and phytoanticipins). Differing in a) molecular weight, b) structure c) functionality and d) class (e.g. alkaloids, flavonoids, terpenoids, phenolics, glycosides, tannins, fatty acids) (Ribera and

Zuniga, 2012). The advantage of plant extracts is that they frequently contain a mixture of chemicals that may work in synergism to inhibit growth of phytopathogenic fungi. Many plant extracts also contain more than one antifungal compound (Masoko and Eloff, 2005). If these compounds have different mechanisms of activity, it may lead to a decrease in the development of resistance. Therefore, the use of plant extracts may curb the development of resistance against antimicrobial compounds.

The naturally occurring compound isolated from *Citrus paradisi*; 7-geranoxycoumarin has good *in vitro* and *in vivo* antifungal activity against *P. italicum* and *P. digitatum*. Also several phenolic compounds (chlorogenic acid, pyrogallol, pyrocatechol, phenol and resorcinol) inhibit *Botryodiplodia theobroma* a causal agent of Java black rot of sweet potato. Kaempferol isolated from *Acacia nilotica*, has antifungal activity against *P. italicum*. The aqueous plant extract enhanced shelf-life of oranges for 6 days (Tripathi and Dubey, 2004). Lawsone isolated from Henna (*Lawsonia inermis*) has powerful fungicide properties (Gurib-Fakim, 2006). Three compounds naphthaquinones and eleutherinone isolated from a chloroform extract of *Eleutherine bulbosa* have antifungal activity at 100 µg/spot. Sesquiterpene and zerumbone have antifungal activity against *R. solani* (Okwute, 2012). Other antifungal compounds from plants have been extensively discussed (Cowan, 1999; Ribera and Zuniga, 2012).

Mahlo and her co-workers demonstrated the potential use of plant extracts to protect plants against fungal pathogens when they investigated the antifungal activity of leaf extracts of several trees against seven plant fungal pathogens. Some extracts had outstanding activity against some of these pathogens. *Bucera bucida* extracts has MICs of 0.02 mg/ml and 0.08 mg/ml against *Penicillium expansum*, *P. janthinellum*, *Trichoderma harzianum* and *Fusarium oxysporum* (Mahlo et al., 2010). *Breonadia salicina* acetone extracts contained at least four antifungal compounds active against plant pathogenic fungi. One of these compounds was ursolic acid (Mahlo et al, 2013). They also showed that crude acetone leaf extracts of *Breonadia salicina* (Rubiaceae) and ursolic acid could protect oranges against infection by *Penicillium* species. Unfortunately the extract was more toxic against Vero kidney cells than against the fungus. The toxicity of this product was too high to use during the transportation of oranges (Mahlo and Eloff, 2014).

Mdee et al. (2009) hypothesized that invasive plant species may be resistant to fungal pathogens because these pathogens may be a limiting factor in determining the spread of plants into other environments. They investigated the antifungal activity of acetone extracts of different parts of seven common South African invasive plant species against selected phytopathogenic fungi (*Aspergillus niger*, *A. parasiticus*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Penicillium expansum*, *P. janthinellum*, *Phytophthora nicotiana*, *Pythium ultimum* and *Rhizoctonia solani* and *Trichoderma harzianum*). All extracts had moderate to good activities on all tested fungi with MICs ranging from 0.08 mg/ml to 2.5 mg/ml. In all cases, leaf extracts were more active than seed or flower extracts. *A. niger*, *P. expansum* and *R. solani* were the most sensitive to all the extracts tested, with average MICs of 0.81, 0.83 and 0.84 mg/ml respectively. The *Campuloclinium macrocephalum* leaf extract was most active against *Colletotrichum gloeosporioides* with an MIC of 0.05 mg/ml.

A potentized leaf extract of *Melianthus comosus*, a weedy plant growing in South Africa, had higher antifungal activity than six commercial fungicides against several important plant fungal pathogens (Eloff et al., 2007). Shuping (2016) followed up on this work and found that several endophytes present in *Melianthus comosus* and *M. major* leaves also had good antifungal activity against the fungal pathogens. It is therefore possible that the compounds responsible for the activity may be produced by endophytes present in the plant. Endophytes may therefore contain interesting antimicrobial compounds that can be effective against plant fungal pathogens. This is because many of the endophytes can assist plants with synthesizing antifungals and protect them against invading plant fungal pathogens (Strobel and Daisy, 2003).

Discussion

It is clear that fungi cause enormous problems in the plant production industry and that inadequate control can lead to serious problems in food production. Phytopathogens do not influence only food and floricultural production but in the medicinal plant industry fungi can also affect the production and the safety of the medicinal plant after harvesting. Existing control measures are not enough to deal with emergence or outbreaks of plant fungal pathogens (Ishii, 2006; Possiede et al., 2009). Therefore, continued research, including using plant based products, is required to provide effective biological products that are cheap, less toxic and effective (Martinez, 2012). Control by using plant based product may offer relief in the fight against plant fungal diseases (Tripathi and Dubey, 2004). Although much research has been done on screening plants for their antifungal activity against phytopathogens, only few secondary metabolites have been isolated (Cowan, 1999). One approach would be to identify new antifungal compounds from plants, but the other possibility is that a complex plant extract can be used. The latter approach has the advantage that there may be reduced development of resistance if the different antifungal compounds in an extract target different receptors. There is however a disadvantage compared to using a single chemical product in ensuring good quality control and variation of activity based on genetic or environmental factors.

Therefore thorough methods have to be used to find new antifungal products that may be of potential use in agricultural production. We also have to investigate how plants which may be of no use in agricultural and horticultural

production protect themselves against pathogen. They may produce novel compounds to overcome pathogen invasion.

Conclusion

There have been many publications on using plant products in human or animal medicine; research on plants that can protect plants against fungi is a productive and important area of research.

While using plant products to deliver organically produced plants or medicine is an important field it will not replace chemical antifungals in the near future. The cost of producing plants, harvesting and extraction is exacerbated by quality control aspects. Using plant-based products could however play an important role in agriculture to treat plant during the withdrawal period before harvesting to allow the concentration of chemical control agents to decrease to safe levels.

While there have been many publications on using plant products in human or animal medicine, research on plants that can protect plants against fungal pathogens or against production animals may be a productive area of research to increase the quality of life of rural people.

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