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Antifungal activity of plant and compost extracts and preparations based on tree bark

Antifungalno djelovanje biljnih i kompostnih ekstrakata i pripravaka na bazi kore drveća

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ABSTRACT

The escalating global demand for food, propelled by population growth, necessitates increased use of plant protection products. However, heavy reliance on chemical pesticides poses sustainability challenges due to their persistence in the environment and potential toxicity to humans and wildlife. To address these concerns, there is a growing shift towards sustainable and eco-friendly plant protection measures, notably biological preparations. This paper explores the potential of alternative methods, particularly biological preparations, for plant protection. It delves into three main categories: plant extracts, compost extracts, and tree bark-based preparations. Plant extracts, derived from sources like nettle, brown algae, and horsetail, exhibit promising antifungal and antibacterial properties, bolstering plant resistance against pathogens. Compost extracts, rich in diverse microbial populations, offer soil enhancement and disease suppression benefits. Additionally, preparations based on tree bark, abundant in phenolic compounds, demonstrate antimicrobial effects against various pathogens. Although some biological preparations are more effective than chemically produced ones, continued research and development of these alternatives are needed to ensure their effectiveness and availability.

Keywords: allelopathy, antagonism, bacteria, biological control, fungi

SAŽETAK

Rastuća globalna potražnja za hranom, potaknuta rastom populacije, zahtijeva povećanu upotrebu sredstava za zaštitu bilja. Međutim, velika ovisnost o kemijskim pesticidima postavlja izazove održivosti zbog njihove perzistencije u okolišu i potencijalne otrovnosti za ljude i životinje. Kako bi se riješili ovi problemi, sve veći naglasak stavlja se na održive i ekološki prihvatljive mjere zaštite bilja. Ovaj rad istražuje potencijal alternativnih metoda, posebno bioloških pripravaka, za zaštitu bilja. Detaljno se razmatraju tri glavne kategorije: ekstrakti biljaka, ekstrakti komposta i pripravci na bazi kore drveta. Ekstrakti biljaka, dobiveni iz primjerice koprive, smeđih algi i preslice, pokazuju obećavajuća protugljivična i protubakterijska svojstva, jačajući otpornost biljaka na patogene. Ekstrakti komposta, bogati raznolikom populacijom mikroorganizama, poboljšavaju svojstva tla i inhibiraju razvoj patogena. Nadalje, pripravci na bazi kore drveta, bogati fenolnim spojevima, imaju protumikrobna svojstva protiv brojnih patogena. Iako su pojedini biološki preparati učinkovitiji od kemijski proizvedenih, potrebna su daljnja istraživanja i razvoj ovih alternativa kako bi se osigurala njihova učinkovitost i dostupnost.

Ključne riječi: alelopatija, antagonizam, bakterije, biološka kontrola, gljive

INTRODUCTION

Given the increasing need for food, driven by the growth of the population on Earth, there is also a growing demand for plant protection products. In practice, chemical preparations are still the most commonly used. However, relying solely on chemical control measures in production is not sustainable in the long term, especially considering the increasing restrictions on their use due to documented harmful effects on the environment and the health of humans and animals (EUR-Lex, 2009; EEA, 2023). The issue with chemical preparations lies in their nature as "foreign bodies" in the environment due to their synthetic origin. Degradation of pesticides can result in compounds that exhibit lower, equal, or even greater toxicity compared to the original pesticides. For example, this is observed in the breakdown of DDT into compounds such as DDD and DDE (Hassaan and El Nemr, 2020). Consequently, they accumulate in ecosystems, including soil and water. Many chemical substances in their composition are toxic to humans and animals. Residues of pesticides are often ingested into the body through food and water, accumulating in the fatty tissues of humans and animals. Their negative impact on aquatic organisms has been documented in numerous studies (Li et al., 2020; Fang et al., 2022). Therefore, finding sustainable and environmentally friendly plant protection measures becomes crucial. There is an increasing emphasis on the use of alternative methods for plant protection. The term "alternative methods" is mainly associated with the application of various biological preparations. Biological preparations encompass a wide range of products, including microorganisms, plant extracts, pheromones, and other natural substances used for pest, disease, and weed control. Biological preparations often contribute to agro-biological sustainability and the reduction of negative environmental impacts (Archana et al., 2022). The application of these preparations can support the creation of a more sustainable agricultural system by promoting balance in soil ecosystems and supporting beneficial organisms. As Sánchez-Hernández et al. (2022) point out, the European Union encourages the use of formulations based on natural products in an integrated pest management context through various regulations. Recent research emphasizes the potential use of various plant and compost extracts, as well as bark-based preparations, which show promise in plant disease control (Verrillo et al., 2021; Behiry et al., 2022). The following section lists such preparations that demonstrate high potential for controlling plant pathogens.

PLANT EXTRACTS

For certain plant extracts, it is known that they do not act as direct or indirect pesticides, but rather they strengthen the plant, making it more resistant to attacks by pathogens. Among the most well-known are preparations based on nettle (*Urtica dioica* L.), brown algae (*Ascophyllum nodosum*), and horsetail (*Equisetum arvense* L.).

Nettle plant extract

Nettle (syn. great nettle, *Urtica*) is a perennial herbaceous plant belonging to the *Urticaceae* family, often categorized as a weed in intensive farming practices (Di Virgilio et al., 2015). In 2017, *Urtica* spp. received approval for use in plant protection by the Commission Implementing Regulation (EU) 2017/419. It is authorized as a fungicide for application on *Brassicaceae*, cucurbits, fruit trees, grapevines, potatoes, tomatoes (for field use), as well as field and protected cucumbers, and both field and protected roses and ornamental trees (EFSA, 2021). Nettle plant extract, or so-called nettle brew, has a proven antifungal effect on numerous phytopathogenic fungi, such as *Colletotrichum* spp., *Diplodia corticola* A.J.L. Phillips, A. Alves & J. Luque, and *Phytophthora cinnamomi* Rands (Ferreira and Oliveira, 2021). In the study by Hadizadeh et al. (2009), ethanolic nettle extract successfully inhibited the growth of *Rhizoctonia solani* J.G. Kühn, *Fusarium oxysporum* Schltdl., *F. solani* (Mart.) Sacc., and *Alternaria alternata* (Fr.) Keissl. Of the species listed, it was most effective against *A. alternata*, where it completely inhibited mycelial growth at a concentration of 0.9%. Behiry et al. (2022) investigated the possibility of using nettle extract and leaf extract of *Dodonaea viscosa* Jacq. in the control of *A. alternata* isolated from tomato.

An antifungal effect was found with growth inhibition values of up to 56% with *D. viscosa* treatment and 54% with *U. dioica* treatment. The authors note that phenolic compounds, including coumaric, caffeic, and ferulic acids, and alpha-tocopherol play a key role in controlling pathogenic fungi. According to EFSA (2021), nettle extract is a complex mixture of chemical substances and the compounds believed to be involved in its biological activity include acetic acid, chlorogenic acid, formic acid, lecithin, L-prunasin, and rutin.

Sea-algae

Sea-algae extract has been approved under Regulation (EC) No 1107/2009, in accordance with Commission Implementing Regulation (EU) No 540/201112 (EFSA, 2012). Brown algae (*A. nodosum*) extract contains trace elements, vitamins, and growth hormones. Examples of such preparations include Algadul®, a liquid extract of marine algae that promotes the stimulation of plant cellular processes and slows down ageing processes in plants (Proeco, 2024), and Bio Plantella (Figure 1), which is listed as an organic fertilizer for use in fruit growing, viticulture, and horticulture (Ministry of Agriculture, 2013).

Brown algae is a rich source of various bioactive phenolic compounds such as phlorotannins and unique polysaccharides (Moreira et al., 2017; Montes et al., 2021). Its antibacterial effect on food spoilage bacteria has been proven, including *Bacillus cereus* Frankland and Frankland, *Escherichia coli* (Migula) Castellani and Chalmers, *Salmonella enterica* subsp. *enterica serovar* Enteritidis (= *Salmonella enteritidis* (Gaertner) Castellani and Chalmers), *Pseudomonas aeruginosa* (Schroeter) Migula, and *Staphylococcus aureus* Rosenbach (Silva et al., 2022). There is potential for research on the impact of brown algae on plant pathogenic bacteria. Van Oosten et al. (2017) state that nearly 47 companies worldwide are currently involved in the production of extracts from *A. nodosum* for agricultural use. *A. nodosum* extract also shows potential antifungal activity. Peres et al. (2012) recorded significant inhibition of *Colletotrichum lagenaria* (Pass.) Ellis & Halst. (= *C. lagenarium*) mycelial growth by *A. nodosum* extract, while no significant inhibition of *Aspergillus flavus* Link mycelial growth was observed simultaneously. Sea-algae extracts do not function via a toxic mode of action and do not present toxicological concerns independently (EFSA, 2012). However, additional risk assessment is necessary for algae from another taxonomic group and for aquatic plants, given that sea-algae extracts act as plant growth regulators (EFSA, 2012).

Figure 1. A preparation based on brown seaweed available for use in Croatia and registered as a plant strengthener (Source: Petrović, E.)

Horsetail

One of the most important plants used for preparing extracts to control fungal pathogens is *Equisetum* spp., a plant species belonging to the *Equisetaceae* family. The most significant *Equisetium* species is undoubtedly horsetail, *E. arvense* (Figure 2), which is the first approved active substance permitted for crop protection in the EU (Marchand et al., 2022). *E. arvense* was approved in 2014 by Commission Implementing Regulation (EU) No 462/2014 for its use as a fungicide (EFSA, 2020). In the study by Trebbi et al. (2021), the horsetail macerate treatment was effective in reducing *Phytophthora infestans* (Mont.)

de Bary (late blight) and increasing yield in tomatoes compared to the untreated control. For durum wheat, the horsetail macerate successfully reduced *Puccinia triticina* Erikss. (brown rust) infection and increased yield under moderate infection conditions, but was ineffective under unfavorable meteorological conditions. In the study by Andreu et al. (2018), horsetail extract inhibited the growth of *Botrytis allii* Munn, *B. cinerea* Pers, and *Penicillium expansum* Link at a concentration of 2000 µg/ml, resulting in reductions of 13%, 23%, and 8% respectively compared to the control. However, it did not inhibit the growth of *Monilinia laxa* (Aderh. & Ruhland) Honey or *M. fructigena* (Pers.) Honey. The primary phytochemical compounds identified in *E. arvense* encompass alkaloids, flavonoids, phenolic acids, phytosterols, tannins, and triterpenoids (Četojević-Simin et al., 2010). The environmental fate and behavior section lacks any evaluation of the possible environmental exposure to *E. arvense* (EFSA, 2020).

E. diffusum D.Don is another *Equisetum* species that has a proven antimicrobial effect. It is rich in phytochemicals with a wide spectrum of activity. Its antifungal effectiveness against fungi *Aspergillus* sp., *Fusarium* sp., and *Nigrospora oryzae* (Berk. & Broome) Petch, and bacterias *Bacillus* spp., *E. coli*, *Salmonella* sp., among others, has been established (Amin Mir et al., 2021).

Figure 2. Preparation based on horsetail available for use in Croatia and registered as a plant strengthener (Source: Petrović, E.).

Other plant extracts

The antimicrobial effect has also been observed with the application of extracts from ragweed (*Ambrosia artemisiifolia* L.) leaves, moso bamboo (*Phyllostachys pubescens* (Carrière) J. Houz.), olive (*Olea europaea* L.), and numerous other plant species (Kleef and Salman, 2022; Liao et al., 2021; Muzzalupo et al., 2020). Muzzalupo et al. (2020) mention the exceptional effectiveness of olive leaf extract in controlling fungi, either in free form or encapsulated in chitosan-tripolyphosphate nanoparticles. Their study recorded a high percentage of inhibition of germination and growth of *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg (87% and 58%). The olive leaf extract demonstrates the ability to exert inhibitory effects on select bacterial species, notably by inhibiting their growth, reducing cellular motility, and impeding biofilm formation (Liu et al., 2017). The olive leaf extract antimicrobial properties are attributed to polyphenols such as hydroxytyrosol, tyrosol, oleuropein, verbascoside, etc. For instance, verbascoside is more effective than oleuropein in inhibiting bacterial growth (Liu et al., 2017). In the previously mentioned study by Andreu et al. (2018), the antifungal efficacy of other plant extracts, such as white willow (*Salix alba* L.) bark, white willow leaves, absinthe (*Artemisia absinthium* L.), and mugwort (*A. vulgaris* L.), was demonstrated. All plant extracts inhibited the growth of *B. allii*, *B. cinerea*, *M. fructigena*, *M. laxa* and *P. expansum* at a concentration of 2000 µg/ml, except for white willow leaves, which did not inhibit the growth of *M. laxa* and *M. fructigena*. The major compounds identified in the extracts were flavonoids and hydroxycinnamic acids. In another previously mentioned research extracts derived from colocynth (syn. bitter apple) (*Citrullus colocynthis* (L.) Schrad), konar (syn. Christ's-thorn) (*Ziziphus spina-christi* (L.) Desf.), and oleander (syn. rose-bay) (*Nerium oleander* L.) inhibited the growth of *A. alternata*, *F. oxysporum*, *F. solani*, and *R. solani*. Colocynth completely inhibited the growth of *A. alternata* and *R. solani*, while konar completely inhibited the growth of *R. solani* at a concentration of 9% (Hadizadeh et al., 2009).

COMPOST EXTRACTS

In recent years, there has been an increase in research related to the potential use of compost in controlling plant pathogens. Organic materials such as manure and compost were previously utilized in agriculture due to their positive impact on improving soil properties and plant health. However, with the advent of chemical fertilizers and pesticides, their application has decreased. This reduced usage and incorporation of organic matter into the soil over the past 50 years has led to a decline in soil structure and health, which has been linked to an increase in plant diseases (Lazarovits, 2001; Bailey and Lazarovits, 2003; Bonilla et al., 2012). Organic materials like manure and compost play a crucial role in preserving soil structure and enhancing its fertility. They contain nutrients and microorganisms that promote soil biological activity, improving its texture, moisture retention, and nutrient-holding capacity. These amendments induce a phenomenon known as soil suppressiveness, primarily targeting fungal pathogens, thereby benefiting farmers financially (Vida et al., 2019). Bonanomi et al. (2010) indicate that the improvement of disease suppression with amendments or compost is frequently specific to particular diseases. Additionally, organic materials help neutralize the harmful effects of chemical fertilizers and pesticides, reducing the risk of soil erosion. Among other benefits, composts increase nutrient levels for plants, thus reducing plant stress and susceptibility to diseases. For example, the application of compost increases the amount of phosphorus in the soil, which has a positive effect on plant growth (Zaccardelli et al., 2013).

In Alfano et al.'s study (2011), the possible antifungal effect of olive waste compost was investigated. The composts contained high levels of active microbial populations that actively grew on chitin and cellulose. During plate inhibition trials, extracts of compost water derived from olive waste exhibited a notable inhibitory impact on the growth of various pathogens, including *Fusarium oxysporum* f.sp. *lycopersici*, *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish (= *Pythium ultimum* Trow), *P. infestans*, *Sclerotinia sclerotiorum* (Lib.) de Bary, and *Verticillium dahliae* Klebahn. Subsequent pot experiments further demonstrated that the application of these composts significantly decreased damping-off caused by *G. ultimum* and wilt disease induced by *F. oxysporum* f.sp. *lycopersici* in tomato seedlings. The authors attributed the inhibitory effect of compost to the antagonistic influence of microorganisms present within it. They found that large populations of aerobic spore-forming bacteria and actinomycetes are present in compost.

Pugliese et al. (2014) state that the inhibitory ability of compost also depends on its physical, chemical, and microbiological composition. Within their investigation, microorganisms isolated from compost derived from green waste and organic household waste underwent assessment for their ability to inhibit pathogens found in vegetable-growing soil. The findings indicated that bacterial strains exhibited notable efficacy in controlling pathogens such as *F. oxysporum* f. sp. *basilici*, *G. ultimum*, and *R. solani*, in comparison to *Trichoderma* sp. and other fungi. Specifically, a total of 74 colonies were isolated from plates and subjected to greenhouse experiments involving potted plants. Among these, three microorganisms (F5, F6, and F7) demonstrated significant disease reduction in the *F. oxysporum* f. sp. *basilici*/basil pathosystem, while another three microorganisms notably decreased root and stem rot induced by *R. solani* on beans (F7, T19, and B17). Additionally, eight other microorganisms, comprising six bacterial strains and two fungi (BL4, BL8, BL15, BL16, BL1A, BL4A, GL1, GK5), exhibited a significant increase in the number of viable plants in the *G. ultimum*/cucumber system and also positively impacted the above-ground biomass of cucumbers.

Suárez-Estrella et al. (2007) isolated 245 bacteria, 175 fungi, and 73 actinomycetes from compost samples, of which initial testing found that 179 strains inhibited the growth of *F. oxysporum* f. sp. *melonis*. The authors emphasize the importance of optimal aeration during the composting process. Isolates with the highest biological activity were mainly identified as *Aspergillus* spp. and were isolated from mature compost samples.

Verrillo et al. (2021) analyzed the bioactive efficacy of compost teas made from agro-industrial residues of artichokes, peppers, and coffee husks to determine their characteristics and potential applications as water-soluble extracts from recycled biomass. Using spectroscopy and gas chromatography/mass spectrometry, the molecular properties of compost teas were determined. Testing the teas' bioactivity on basil seed germination, antioxidant capacity, and antimicrobial activity revealed that compost teas from coffee and peppers showed the highest potential. Seed germination experiments showed no phytotoxic effects, and the application of compost teas resulted in a significant increase in root and hypocotyl growth. The antimicrobial effect was particularly noticeable against Gram-negative bacterial strains such as *P. aeruginosa*, *E. coli*, and *Klebsiella pneumoniae* (Schroeter) Trevisan. The authors highlight that the bioactivity of compost teas is associated with their hydrophobic characteristics and molecular composition, particularly the presence of soluble aromatic compounds such as lignin and phenol derivatives.

TREE BARK-BASED PREPARATIONS

Plant bark is also rich in phenolic compounds that have been documented to have antimicrobial effects. During the 1950s, the nursery industry in the United States and Australia developed cheaper substrate mixes and soil for woody ornamental plants that were based on bark rather than peat, initiating the use of such preparations (Hoitink and Ramos, 2004). Some growers discovered that composted bark could effectively inhibit root rot caused by *Phytophthora* sp., especially in cases where resistant plant varieties or alternative chemical control methods, aside from methyl bromide, were unavailable (Neher and Hoitink, 2022). Fernandes et al. (2011) extracted 33 components from the cork oak bark (*Quercus suber* L.). The most abundant components were derivatives of gallic acid, gallotannins, and ellagitannins. The chemical composition of cork is influenced by the cork harvesting regime, genetic factors, geographic origin, temperature, soil conditions, as well as tree size and age (Touati et al., 2015). Sánchez-Hernández et al. (2022) investigated the *in vitro* antimicrobial activity of an aqueous ammonia extract derived from the cork oak bark against several species including *M. fructigena*, *M. laxa*, *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips, and *Phytophthora cactorum* (Lebert & Cohn) J. Schröt., known causative agents of drying and rotting, as well as the bacteria *Erwinia amylovora* Burrill and *Pseudomonas syringae* pv. *syringae*, either individually or in combination with chitosan oligomers. A potent antimicrobial effect was observed, significantly surpassing that of conventional pesticides such as azoxystrobin and fosetyl AL. The authors attributed the antimicrobial activity of the cork extract to the presence of glycerin and vanillic acid, which were tested as pure compounds. Their research group previously investigated the efficacy of extracts of *E. arvense*, *U. dioica*, and *Silybum marianum* (L.) Gaertn on isolates of *N. parvum*, with antimicrobial effectiveness being lower than that of *Quercus* sp. bark extract. Bark extracts show synergistic effects by inhibiting various, mostly Gram-positive bacteria and have a pronounced radical scavenging ability (Häsler Gunnarsdottir et al., 2023). Karličić et al. (2021) found the antifungal effect of Scots pine (*Pinus sylvestris* L.) bark extract in controlling the pathogens of Botryosphaeria dieback. Extracts from wood industry residues, such as white birch (*Betula papyrifera* Marshall) bark extract, can be applied to suppress certain microorganisms (Blondeau et al., 2020). In Blondeau et al.'s (2020) study, the water extract inhibited the growth of eight species of microorganisms at low concentrations. The authors identified catechol as one of the main components in the water extract, suggesting that this component could be one of the most important contributors to the antimicrobial activity of the extract. They also highlight the possibility of utilizing wood industry residues. However, Jiang et al. (2018) conducted a study in which catechol was added to the nutrient medium, and they found that the presence of catechol led to a reduction in the activity of the antioxidant enzymes of the fungus *R. solani*, including catalase and peroxidase, but also led to a significant increase in the activity of plasma glutathione peroxidase by 310% at 50 µg/mL of catechol. In conclusion, they found that the growth rate of the fungus was reduced, but the pathogenicity (for rice) increased in the presence of catechol.

LIMITING FACTORS IN THE APPLICATION OF PREPARATIONS

Numerous antagonistic organisms naturally present in plant and compost preparations can be pathogenic to the plant on which they are applied. For example, Khatri et al. (2023) mention the possibility of using the fungus *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. to control *Fusarium odoratissimum* Maryani, L. Lombard, Kema and Crous. However, *C. gloeosporioides* is a pathogen with a wide host range and is widespread worldwide. Dean et al. (2012) list this species among the top 10 most significant plant pathogens globally. Additionally, there is a risk of introducing pathogens that can affect both animals and humans. Another issue is the unavailability of preparations on the market; most products are registered as plant strengtheners and are much more expensive than chemical preparations. The effectiveness of biological preparations often depends on specific environmental and climatic conditions, making them less effective than chemical preparations. Farmers and users of these products often lack relevant information about biological preparations and their mode of action, resulting in inadequate application and significant reduction in efficacy. Biological preparations usually require more time to achieve the desired effect compared to some chemical pesticides. This can be problematic when a quick reaction is needed to prevent serious damage to crops. Bonilla et al. (2012) emphasize in their review paper that despite the abundance of information regarding the positive effects of organic amendments in inhibiting the growth of phytopathogens, challenges persist in their practical applicability. For instance, the direct utilization of natural substances may enhance soil characteristics, yet it also carries potential hazards due to the utilization of intricate remnants that might harbour harmful elements or result in an overabundance of phosphorus, potassium, and nitrates (Rivero et al., 2004).

CONCLUSIONS

The increasing demand for food due to population growth presents a challenge to agriculture, particularly in plant protection. Chemical pesticides traditionally used for this purpose, although effective, leave serious ecological and health consequences. Therefore, the search for sustainable and environmentally friendly methods of plant protection becomes imperative. Biological control, especially the application of biological preparations, represents a promising approach to combating plant pathogens. Plant extracts and preparations based on tree bark provide support to plants by enhancing their natural resistance. Compost extracts, due to their diverse microbiological population, contribute to soil improvement and reduce plant susceptibility to diseases. The vast majority of biological preparations rely on the antagonistic potential of microorganisms, but also on the composition of phenols in the preparations. However, the mechanism of action of biological preparations has not yet been fully explored. Despite achievements in research, continued research and development of these alternatives are needed to ensure their effectiveness, economic viability, and broader availability. Furthermore, collaboration between scientists and farmers is crucial for the successful implementation of biological methods in agriculture.

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