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[1] Department of Plant Protection, Faculty of Horticulture and Landscape Architecture, University of Life Sciences in Lublin, Leszczyńskiego 7, 20-950 Lublin, Poland

[2] Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco delle Scienze 11/A, 43124 Parma, Italy

*e-mail: weronika.kursa@up.lublin.pl

AGNIESZKA JAMIOŁKOWSKA [1]*, WERONIKA KURSA [1]*, FRANCESCA DEGOLA [2]

Plant origin preparations – an eco-friendly tool of modern strategies for plant protection against fungal pathogens

Preparaty pochodzenia roślinnego – ekologiczne narzędzie nowoczesnej strategii ochrony roślin przed grzybami chorobotwórczymi

Summary: Plants are a valuable source of many bioactive compounds. Numerous scientific studies confirm the antimicrobial effect of plant extracts against many phytopathogens, including pathogenic fungi. Currently, the attention is mainly focused on the production of preparations of plant origin containing stable and biodegradable biologically active compounds to control plant diseases. They are also an alternative to the conventional method of protection against pathogens. This review includes the characteristics of the most popular herbal plants (tansy, yarrow, garlic, horseradish, nettle) and the bioactive compounds contained in them, as well as the possibility of their use in plant protection, especially for control of pathogenic fungi.

Key words: plant extracts, pathogenic fungi, bioproducts, IPM

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INTRODUCTION

Feeding humanity, and thus food production, is one of the greatest challenges for the modern global economy. New European strategies and the concept of sustainable agriculture force a reduction in the use of pesticides and synthetic fertilizers [Mołoń and Durak 2018]. Preparations of natural origin, which can partly replace synthetic phyto-pharmaceuticals, are becoming an alternative to current agriculture [Grzyb et al. 2019, Jamiołkowska 2020]. Pesticides, despite their widespread availability, ease of use, and high effectiveness, carry a number of hazards, mainly to the natural environment and human health [Mickiewicz and Mickiewicz 2014]. Many synthetic chemical preparations have a negative effect on beneficial organisms and accumulate in plant tissues over long periods of time. Pursuant to Directive 2009/128/EC of October 21, 2009, Poland, like other European Union Member States, was obliged to implement the principles of integrated pest management (IPM) from January 1, 2014 [Pruszyński et al. 2008, Sosnowska et al. 2016]. The new development strategy of the European Union – "European Green Deal"– assumes the implementation of the concept of sustainable development, and thus stopping climate change and transforming the economy towards a circular model [Parlińska et al. 2020, Montanarella and Panagos 2021]. One of the goals is the need to limit the use of chemical means of production by reducing the application of chemical pesticides by 50% by 2030, with particular emphasis on reducing the most hazardous pesticides, as well as the use of chemical fertilizers by 20% [Montanarella and Panagos 2021].

Integrated pest management is a protection system whose main objective is to primarily apply non-chemical methods. As a result, chemical treatments are limited and environmental risks are minimized [Pruszyński et al. 2008]. Integrated pest management is a component of integrated crop production technology, which treats the entire farm as a basic unit, i.e. applies a holistic approach to the system [Pruszyński et al. 2008]. Monitoring, signaling, as well as pest and disease prediction and prevention are key components of this system [Sosnowska et al. 2016]. Among the benefits of using an integrated protection system is an increase in the biodiversity of agrocenoses, as well as higher social awareness of both consumers and producers. This allows for the production of safe food, free from pesticide residues, nitrates, heavy metals and other harmful substances. The use of pesticides and environmental pollution are reduced thanks to the principles of good plant protection practice [Sosnowska et al. 2016].

Synthetic chemicals that over decades have been widely used for plant protection are nowadays often replaced by products of natural origin that includes humic acids, microbial consortia and botanicals, as their applications provide a number of documented benefits to plants in both stimulating growth and enhancing defenses against biotic and abiotic stresses (for example through priming mechanisms on plant immunity) [D'Addabbo et al. 2019, Zulfiqar et al. 2020]. Even if their efficacy largely depends on their composition, preparations of natural origin are preferred in sustainable eco-friendly agriculture and recommended by various national and supranational Institutions such as the Official Journal of the European Union [Directive 2009/128/EC], the National Organic Program USDA [USDA 2017], and the Organic Materials Review Institute [https://www.omri.org/omri-lists].

This review focuses on the characterization of the most popular herbaceous plants and the bioactive compounds they contain in terms of their effect against phytopathogens, with a particular emphasis on pathogenic fungal species. In addition, the mini-review describes the possibilities of their application in the production of biological preparations as elements of modern strategies for plant protection.

BIOLOGICAL METHOD OF PLANT PROTECTION

The biological approach is a very important component of non-chemical protection methods, which involves the use of beneficial organisms (bio-preparations) and other preparations of natural origin (biotechnical preparations) to control crop pests (Fig. 1) [Sosnowska 2018]. The specific purpose of biological protection, in addition to the use of natural preparations, is to take advantage of self-regulatory processes occurring in the environment and their subsequent incorporation into the technological process of plant production. The biological method is potentially safer for plants and native microflora, which is relevant for soil cultivation because it scarcely affects the rhizosphere microbiota [Sosnowska 2018]. Preparations of natural origin are characterized not only by high efficacy, but also by an almost null toxic effect on consumers, that, in turn, are even more prone to prefer food commodities and derivatives obtained with organically managed systems. Among them, preparations based on plant extracts with antimicrobial (antibacterial and antifungal) activity play an increasingly important role in the global market of commercial products (Tab. 1) [Jamiołkowska 2013, 2020, Mołoń and Durak 2018, Krzepiłko et al. 2020].

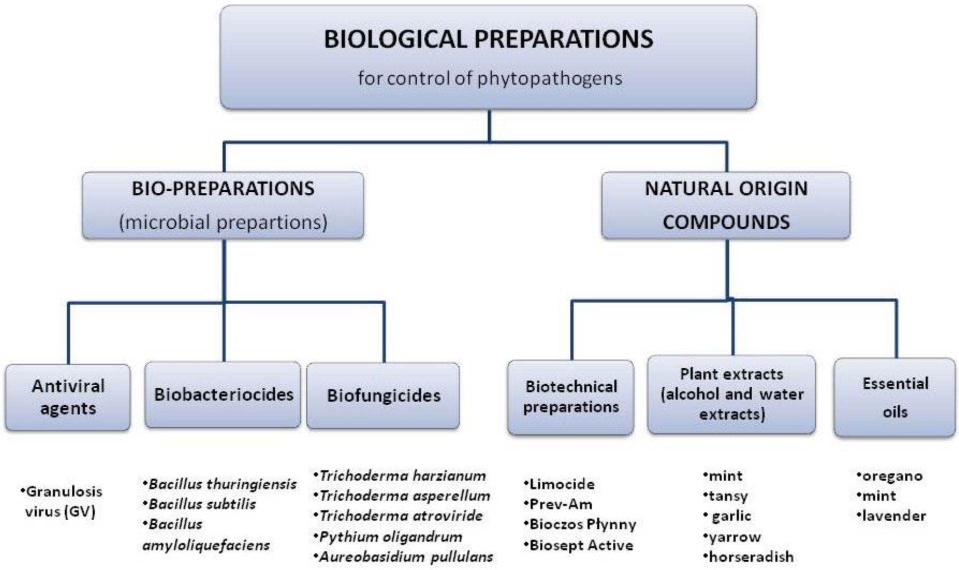

Fig. 1. Classification of biological preparations for control of phytopathogens

Table 1. Commercial preparations of plant origin used in crop protection

Commercial name of the preparation	Active ingredient	Plant species	Preparation type	Manufacturer	References
Timorex Gold 24 EC	tea tree oil	*Camelia sinensis*	fungicide	BIOMOR EUROPE Ltd., UK and Northern Ireland	Szymona 2010, Martyniuk 2017
Biosept Active	grapefruit pulp and seed extract	*Citrus paradisi*	fungicide	CINTAMANI, Poland	Heller et al. 2010, Świerczyńska 2010, Jamiołkowska 2013
Bioczos Płynny, Bioczos BP	garlic pulp extract	*Allium sativum*	fungicide, repellent	HIMAL Łódź, Poland	Szymona 2010, Świerczyńska 2010, Jamiołkowska 2013
Prev-Am, Prev-Bio	orange oil	*Citrus* spp.	fungicide, insecticide, acaricide	ORO AGRI EUROPE SA, Portugal	Kempka 2014
Limocide	orange oil	*Citrus sinensis*	insecticide, acaricide, fungicide	VIVAGRO, France	Rezende et al. 2020

ANTIFUNGAL POTENTIAL OF SOME COMMON PLANT EXTRACTS

Plants used in folk medicine are also applied in crop protection. Currently, increasing importance is attributed to wild-growing herbaceous plants: in fact, due to the synergic action of many secondary metabolites that have been evolved by plants to cope with pathogens and pests, as well as with environmental stressors, wild species not subjected to human selection exhibit greater resistance to biotic and abiotic stresses [Mundy et al. 2016]. Thus, these species have been represented important sources of natural pesticides and drugs for centuries [Villaverde et al. 2016]. In recent decades, antimicrobial and antifungal properties of various extracts and their components, e.g. essentials oils, have been studied and attention is focused on the application of these natural materials in alternative plant protection products, as they are biodegradable and non-toxic to the environment [Šernaité 2017]. The most popular, also occurring in the natural environment, including Poland, are tansy, St. John's wort, yarrow, sage, garlic, nettle, hops, horseradish [Nawrot et al. 2020, Kursa 2022]. M any bioactive compounds have been isolated from plants, and some of them have contributed to the development of novel plant-based biopesticides used in food production [Kursa et al. 2022], but an appropriate selection of biomolecules for the formulation of biopesticides with multiple modes of action against target pathogens is critical to provide a safer alternative for sustainable and organic production [Jamiołkowska 2020]. Biological activity and functioning mechanisms of plant compounds are variable, and many of them are not yet fully understood yet. Plant extracts consist of a plethora of compounds specific to the plant from which they were extracted and its phonological/developmental status, and belong to the most various chemical classes. Aromatic secondary metabolites synthesized by plants and recognized as potent antimicrobials include alkaloids, phenolic acids, quinines, flavones, flavonoids, tannins and coumarins [Šernaité 2017], but also peptides, hormones, saccharides, lipids, vitamins and nitrogen- and sulfur-containing compounds have been proven to exert biological activity against fungi [Commisso et al. 2021].

Yarrow (*Achillea millefolium* L.) is a herb found in the wild in many regions of the world. It is common in North America, Asia and Europe and can inhabit lowland and mountain areas [Derda et al. 2012]. The plant is widely applied in traditional medicine, due to a variety of pharmacological activities from antibacterial to anti-inflammatory, from antioxidant to anticancer properties that rely on a wide range of bioactive compounds [Dall'Acqua et al. 2011, Bączek et al. 2015, Verma et al. 2017, Afshari et al. 2018], but the specific antifungal potential of yarrow extracts against *Aspergillus niger*, *Candida albicans*, *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum coccodes* and *Fusarium oxysporum* in particular has been reported [Candan et al. 2003, Kursa et al. 2022]. These and other antimicrobial activities are due, among others, to the high flavonoid content of its leaf extracts [Vitalini et al. 2011, Kaczorová et al. 2021, Kursa et al. 2022], that was demonstrated to be higher than in flowers [Keser et al. 2013]. Naringin (a flavanone-7-O-glycoside) in particular is the main compound from the flavonoids group present in yarrow, that beside possessing well-acknowledged anti-proliferative and anti-inflammatory activity also shows antifungal properties [Salas et al. 2011], but essential oils also contribute to both yarrow's antifungal and antibacterial properties [Rahimmalek et al. 2009, Bączek et al. 2015].

Tansy (*Tanacetum vulgare* L.) is an important herbaceous plant and a component of many biocidal preparations. Its natural habitat is the northern hemisphere, and its range covers Europe and the temperate zone of Asia [Derda et al. 2012], being also accidentally introduced in North America, East Asia and some Arctic countries. For centuries, tansy has been widely used in folk medicine, mainly to repel insects [Derda et al. 2012], but the plant is a valuable source of biologically active substances [Coté et al. 2017] such as tannins, organic acids and sugars, sesquiterpene lactones, flavonoids and essential oil [Zawiślak and Nurzyńska-Wierdak 2017] potentially exploitable for other agricultural purposes. However, should be mentioned that tansy is a toxic plant, and can be poisonous even in small doses [Vilhelmova et al. 2020]. Due to its rich chemical composition, this species exhibits antibacterial, anti-inflammatory, antioxidant, and cytotoxic effects [Coté et al. 2017, Ivănescu et al. 2018], as well as antifungal activity, even if its effect on microorganisms greatly depends on the chemical composition of the essential oil [Devrnja et al. 2017]. In fact, not only flavonoids, but also essential oils and their composition determine the antimicrobial activity of tansy extracts [Vilhelmova et al. 2020]. The chemical composition of the essential oils of this plant varies depending on the geographical location, soil composition, its quality, and meteorological conditions [Mot et al. 2018], but sesquiterpene lactones (STL) are an extremely important group of compounds contained in tansy essential oils [Vilhelmova et al. 2020]. Ferulic acid, rosemary acid, chlorogenic acid, and the flavonoid luteolin have also been detected in the ethanol extract of yarrow. Studies have shown that tansy exerted a strong fungistatic and fungicidal effect, especially in methanol extracts, on the following fungi: *Trichoderma viride*, *Aspergillus fumigatus*, *A. niger*, *A. ochraceus* and *A. versicolor*, as well as *Penicillium funiculosum*, *P. ochrochloron* and *P. verrucosum* var. *cyclopium* [Devrnja et al. 2017]. A strong fungistatic effect against Alternaria alternata (PCL10) and Botrytis cinerea (CH10) was found for a 20% tansy extract. It reduced the surface growth of Colletotrichum coccodes and Fusarium oxysporym by 17.98–24.70% in the first days of treatment [Kursa et al. 2022]. This study proved that the fungistatic effect of tansy was related to the high content of flavonoids (22.27 mg/mL) and polyphenols (36.85 mg/mL) in the extract [Kursa et al. 2022]. Consistently with Mot et al. [2018] tansy is a plant with a high phenol contents, which confirms its high antioxidant activity. Research by Ivănescu et al. [2018] showed that chlorogenic acid was the main phenolic acid found in tansy plants collected in Romania, while Bączek et al. [2017] reported chicoric acid to be dominant in other individuals, confirming the diversity of the chemical composition of bioactive compounds depending on the geographical area. High content of polyphenolic compounds in alcoholic extracts of herbaceous plants is usually correlated with high scavenging capacity, as obtained with the DPPH free radical method. Study on methanol extracts of tansy showed the strongest antioxidant activity correlated with the highest phenolic content [Devrnja et al. 2017], as well as previous research that an antioxidant potential of yarrow extract (82.14% DPPH inhibition) was the highest if compared to extracts from other plant [Fierascu et al. 2015].

Garlic (*Allium sativum* L.). Another plant rich in biologically active compounds is common garlic. It is native to Asia Minor, but it is commonly found in temperate areas [Dębski and Milner 2007]. Cultivated not only for its taste but also as a medicinal plant, this species has been widely used for thousands of years [Harris et al. 2001]. In fact, many studies have confirmed the multidirectional biological garlic properties, that namely are antiviral, antibacterial and antifungal activities [Dębski and Milner 2007, Jamiołkowska 2013, Nawrot et al. 2020]. Antioxidant and antigenotoxic effects of garlic extracts were also confirmed [Park et al. 2009]. The antifungal activity of garlic pulp as the main ingredient of the Bioczos Płynny preparation has been confirmed by Jamiołkowska [2013], therefore this preparation is recommended for the protection of weet peppers grown in the field against pathogenic fungi. The antimicrobial activity of garlic is attributed to allicin (diallyl thiosulphinate), a sulfur-containing molecule formed by the conversion of alliin by allinase enzyme; because of its prominent antifungal effects allicin has been actively investigated, showing to inhibit both the germination of spores and the growth of hyphae produced by *Candida*, *Cryptococcus*, and *Trichophyton* species. The antimicrobial effects of allicin are related to its ability to strongly inhibit thiol-containing enzymes such as cysteine proteinases, alcohol dehydrogenases, and thioredoxin reductases [Akira et al. 2005].

Horseradish (*Armoracia rusticana* L.) is another plant worth attention. It contains substances characterized by bactericidal or bacteriostatic properties, thanks to which they inhibit the development of harmful microflora [Ratajczak et al. 2017, Manuguerra et al. 2020]. Horseradish plant extracts are considered to be one of the most active against microorganisms. Biologically active compounds contained in horseradish plants are characterized by significant antimicrobial activity and cytotoxicity [Park et al. 2009, Plaszkó et al. 2021], such asallyl isothiocyanate, the compound pointed as mainly responsible for the antibacterial effect of horseradish [Park et al. 2009, Jamiołkowska 2013]. Detailed chemical analysis revealed the presence of numerous glucosinolates and glycoalkaloids also [Agneta et al. 2013]. Research conducted by Kursa et al. [2022] showed that horseradish leaf extract inhibited in vitro the surface development of *B. cinerea*, *C. coccodes* and *F. oxysporum* only when used at a 20% concentration (16.27–53.57% inhibition), but almost no fungistatic effect at lower doses. Tedeschi et al. [2011] reported that horseradish in 10% ethanol solution showed fungistatic activity only against *F. oxysporum*, *F. culmorum* and *Sclerotium rolfsii*, while it did not inhibit the growth of *B. cinerea* and *Trichoderma longibrachiatum*. Amongst the highly bioactive glucosinolates (GLs) and their degradation products found in this species, sinigrin is the dominant in roots and leaves [Agneta et al. 2013]. Although glucosinolates often do not directly inhibit the fungal growth, their degradation products (metabolites), which include the highly volatile isothiocyanates, show strong antifungal and antibacterial activity instead, being able to affect membranes and cell structures through electrophilic reaction towards thiol groups of proteins, peptides and amino acids [Biller et al. 2019, Plaszkó et al. 2021]. This indicates that they can play an important role in plant resistance to fungi, nematodes and other pathogens [Stoin et al. 2007]. Rutoside and quercetin are the main flavonoids present in A. rusticana [Jafernik et al. 2019], and they exhibit antiviral, antimutagenic, antioxidant and anticarcinogenic properties [Petrović et al. 2021]. Other phenolic compounds found in horseradish include gallic and tannic acids [Jafernik et al. 2019]. It is noteworthy that Tomsone et al. [2020] showed that microcapsules from the juice of horseradish leaves showed a higher content of phenolic compounds and stronger antioxidant activity than those from the roots. At the same time, horseradish root and leaves are rich in polyphenols [Petrović et al. 2021, Kursa et al. 2022]. Numerous studies also confirmed the strong scavenging activity of horseradish extracts [Jafernik et al. 2019].

Stinging nettle (*Urtica dioica* L.) is a wild herbaceous plant naturally occurring in Asia, Europe, North America and North Africa [Bhusal et al. 2022]. Nettle owes its popularity not only to its widespread distribution, but also to its remarkable exploitation as a plant with anti-inflammatory, antimicrobial and antioxidant effects [Altamimi et al. 2022, Tarasevičienė et al. 2023]. The main bioactive components contained in the plant include flavonoids and phenolic acids (hydroxybenzoic acid and cinnamic acid derivatives), amino acids, carotenoids, organic acids and fatty acids [Devkota et al. 2022]. The pharmacological activity of nettle results from the presence of phenolic compounds found in the leaves, where the highest accumulation of these compounds occur [Altamimi et al. 2022]; additionally, it has been shown to be richer in individual polyphenols than other wild plants. Rutin (flavonoid-3-O-glycoside, also known as quercetin 3-O-rutinoside) in particular is considered to be the main bioactive phenolic compound, being a highly potent antioxidant molecule [Vajić et al. 2015]. However, as for other plants, different factors affect the chemical composition of nettle organs, such as the variety, genotype, climate, soil, vegetative stage, harvest time and treatment: for example, not only polyphenolic acids content was described higher in male individuals, but also their chemical profiles were deeply different from the female. Accordingly with such bioactives pattern, the antifungal potential of *Urtica* species has been reported: the fungistatic effect of aqueous extracts of nettle apices was documented by Burgieł [1995] on *B. cinerea* and *Colletotrichum lindemuthianum* colonies, while methanolic leaf extracts of *U. dioica* were found to be effective against *Alternaria alternata* [Behiry et al. 2022].

PLANT ORIGIN PREPARATIONS AS AN ELEMENT OF MODERN PLANT PROTECTION

Herbs can be used to protect plants against pathogens in the form of commercial products as well as self-prepared decoctions, infusions or extracts. However, extreme caution should be exercised, as some extracts used for spraying can be phytotoxic (e.g. wormwood or horsetail extract) and are quickly biodegraded due to the low stability of the biologically active compounds they contain. However, many organic compounds have already been commercialized and are present on the market in the form of biotechnical preparations. These products are biodegradable, non-toxic and do not pose a threat to various non-target organisms. In addition, many of them not only directly limit the development of phytopathogens but can also induce enhanced immunity that can also alleviate stress-induced limitations by regulating/modulating plant physiological processes (Fig. 2) [Yakhin et al. 2017, Jamiołkowska 2020]. The growing demand of the phytosanitary market for non-harmful to human and the environment products and the limitations of conventional methods of obtaining and preparing plant-derived bioproducts, motivate the continuous search for more efficient production methods of useful, biologically active compounds from plant material. Such an opportunity is represented by the *In vitro* culturing of plant cell lines and tissues, as they possibly allow for a better efficiency of biosynthesis of valuable, selected, tailored metabolites [Kawka et al. 2017]. In fact, the production of interesting bioactives compounds by using plant biomass grown *in vitro* allows for strict control of the conditions of the technological production system. This method allows to obtain biologically stable as well as pure compounds and is an economically justified alternative to conventional protection methods. In the future, the new technology will contribute to the development of pure and stable compounds and new bioproducts for widespread use in agriculture [Villaverde et al. 2016]. Some of them can even be used in mixtures with pesticides, which does not affect their effectiveness while improving yield [Sultana et al. 2011].

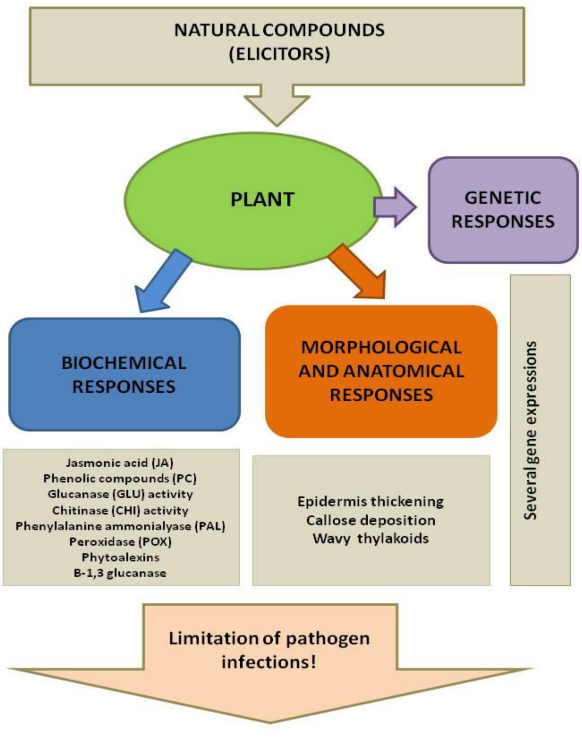

Fig. 2. Reaction of plants under the influence of preparations of plant origin as elicitors [Jamiołkowska 2020]

CONCLUSIONS

The use of natural plant-derived compounds to control phytopathogens is very attractive, and the availability of new applications and molecular techniques is opening up new approaches to crop protection. Due to the phenomenon of pest resistance and the withdrawal of many active pesticide substances from plant production, the preventive use of products based on natural ingredients can and should become an alternative to pesticides. Many plant species contain chemical compounds in their tissues that are released under biotic stress conditions, e.g. attack by pathogenic microorganisms. These include phytoalexins, phytoncides, glycosides, amino acids, polyamines, growth inhibitors and phenolic compounds [Wolski and Gliński 1998]. It has also been proven that plant extracts support the natural resistance of plants to stress factors. Thanks to these properties, they become a potential source of natural fungicides. Due to the fact that the registration of products based on natural components has been facilitated, there are more and more of them on the phytosanitary market. They contain not only plant extracts, but are additionally enriched with microelements and are classified as plant biostimulators. These preparations, due to their antimicrobial effect, low toxicity, rapid biodegradability and lack of residues, are more and more often used in plant protection and the food industry [Burgieł 2005]. The scope of application of the biological method is gradually increasing, and at the same time, it should be noted that the use of biological methods alone as part of the plant protection process is definitely an insufficient measure [Sosnowska 2018]. Therefore, the best solution for plant protection is a rational combination of conventional and biological approaches because in the current situation it is not yet possible to completely stop using synthetic pesticides in plant protection.

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