

Editorial

Biopesticides: Naturally Originating Plant Protection Products and Biocides

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Pesticides are substances that prevent, repel, suppress, mitigate, or eradicate harmful organisms. They include both “plant protection products” (used in agriculture) and “biocidal products” (used to control pests or medically significant bacteria or to protect materials). In recent years, the number of synthetic pesticides has become drastically restricted in the EU because of their environmental side effects and subsequent non-inclusion in Annex I of 91/414/EEC. The small number of resulting commercial pesticidal formulates has led to resistance issues and low efficacy. Thus, there is an urgent need to develop new, effective, and safe pesticides. Biopesticides tend to pose fewer risks than conventional pesticides, and recent advantages in food safety generally associate them with biological control and IPM, methods that aim to reduce the use of synthetic pesticides. Several natural plant secondary metabolites are proven to exhibit significant pesticidal activity and can be prepared in a “green”, easy, and cost-effective way without the use of organic solvents or sophisticated extraction procedures. These crude extracts, composed of a wide variety of constituent secondary metabolites, often act at multiple or novel target sites, reducing the likelihood of resistance development. Some are even proven to inhibit the cytochrome P450 detoxification agents, thus synergically acting with synthetic formulates.

In the context of studying botanicals for their pesticidal properties, Badalamenti et al. explored the insecticidal potential of *Ridolfia segetum* (L.) Moris essential oil (EO) against three different pests: *Culex quinquefasciatus* Say, *Musca domestica* L., and *Spodoptera littoralis* (Boisduval) [1]. In fact, several species of the family Apiaceae produce essential oils that can be used on an industrial scale for pharmaceutical, cosmetic, and food purposes. In this study, the EO was obtained by hydrodistillation of flowers, and its composition was achieved by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). This EO is rich in α -phellandrene (49.3%), β -phellandrene (9.2%), terpinolene (20.7%), and piperitenone oxide (5.9%). Concerning the mosquitocidal efficacy, the EO demonstrated a noteworthy toxicity against *C. quinquefasciatus* 3rd instar larvae, with a $LC_{50} = 27.1 \mu\text{L L}^{-1}$ and $LC_{90} = 42.5 \mu\text{L L}^{-1}$. Regarding *M. domestica*, a different toxicity of the *R. segetum* EO was found on male and female flies, with LD_{50} values of 10.5 and 50.8 $\mu\text{g adult}^{-1}$, respectively. The EO was also toxic to *S. littoralis* 3rd instar larvae, achieving LD_{50} and LD_{90} values of 37.9 and 99.6 $\mu\text{g larva}^{-1}$, respectively. The authors concluded that this flower EO, extracted from a traditional Sicilian food plant, merits further investigation for the development of green insecticide formulations to be used in real-world scenarios, pending a careful assessment of non-target toxicity on beneficial organisms.

However, other than efficacy concerns, it is also of essential to pinpoint the secondary effects of biorational biopesticidal tools on beneficial organisms. In this framework, Monokrousos et al., investigated the effects of three botanicals with nematocidal properties (anise-*Pimpinella anisum*, parsley-*Petroselinum crispum*, and rocket-*Eruca sativa*) on the soil nematode community, in terms of trophic structure and nematode genera composition [2]. They compared the effects with those of fluopyram (synthetic nematicide) and Nemagold (bionematicide). They assessed the role of time by sampling the botanicals’ treatments 15 and 45 days after treatments and analyzing nematode genera and microbial phospholipid



Citation: Ntalli, N. Biopesticides: Naturally Originating Plant Protection Products and Biocides. *Agriculture* **2022**, *12*, 964. <https://doi.org/10.3390/agriculture12070964>

Received: 25 June 2022

Accepted: 30 June 2022

Published: 5 July 2022

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fatty acid biomarkers (PLFA). The incorporation of the botanicals into the soil reduced plant parasitic nematodes and increased bacterivores, especially enrichment opportunists, among them *Rhabditis*, with no effect on fungivores and non-parasitic plant feeders. Neither the number nor the composition and dominance hierarchy of nematode genera were affected. Nemagold did not induce any significant change, while fluopyram decreased both free-living and parasitic nematodes, but with no uniform effect against all genera. The least-affected genus was the fungivorous *Aphelenchus*. While most microbial PLFAs increased with time, the abundances of nematode genera did not change, except for the *Meloidogyne incognita* second stage juveniles, which emerged in soil only 45 days post treatments. The low enrichment index and high channel index values of the fluopyram soil samples indicated a stressful environment. The opposite was observed in the botanical treatments, especially for parsley and rocket.

Natural substances derived not only from plants but also from entomopathogenic fungus species have significant pesticidal attributes. Abdulle et al. reported that a *L. lecanii*-extracted partially purified protein triggered a systemic resistance against *Bemisia tabaci* in the cotton plants, proposing its putative effectiveness as an innovative biological control technique against *B. tabaci* and other phloem-feeding hemipteran pests [3]. In particular, they studied the anti-insect impact of a protein extracted and partially purified from an entomopathogenic fungus (EPF) *Lecanicillium lecanii* (Zimmermann) against *B. tabaci*. Whiteflies, *B. tabaci* Gennadius (Aleyrodidae: Hemiptera) are a polyphagous economically destructive pest of several solanaceous crops around the world. Many secondary metabolites are synthesized by different biotrophic and necrotrophic fungi, which are capable of inducing systemic resistance in plants against various phytophagous pests. Three different concentrations (i.e., 7.43, 11.15, and 22.31 $\mu\text{g mL}^{-1}$) of this protein were bioassayed to assess its effect on the fecundity rate of *B. tabaci* in cotton (*Gossypium hirsutum* L.) plants. Furthermore, the possible implication of this fungal protein in defense pathways of cotton plants was evaluated by determining the expression profiles of salicylic acid (SA) and jasmonic acid (JA) pathways related to major genes through reverse transcription qPCR (RT-qPCR). According to the results, all protein concentrations exerted a significant ($F_{3, 252} = 62.51$; $p \leq 0.001$) and negative impact on the fecundity rate of *B. tabaci* females. At the highest protein concentration (22.31 $\mu\text{g mL}^{-1}$), the minimum rate of fecundity (i.e., 2.46 eggs female $^{-1}$ day $^{-1}$) of *B. tabaci* was noted on the seventh day, whereas fecundity rates for the other two protein concentrations (i.e., 11.15 and 7.43 $\mu\text{g mL}^{-1}$) were 3.06 and 3.90 eggs day $^{-1}$ female $^{-1}$, respectively. The maximum rate of fecundity (6.01 eggs female $^{-1}$ day $^{-1}$) was recorded in untreated (control) treatments. In addition, the foliar application of *L. lecanii*-derived protein significantly upregulated all SA-linked genes (OPR3, PPO1 and COI1) and triggered a slight upregulation of JA-linked genes (LOX1, UBG7 and AOS) in the cotton plants. The authors conclude that further investigations are required, especially the purification and molecular and functional characterization of this *L. lecanii*-derived partially purified protein.

Most interestingly, fungal biopreparations can help also combat resistance since they are reported to synergically act with commercial pesticides, helping the latter to act at lower concentrations than their registered doses. *Penicillium* fungi are found to produce various biologically active compounds with antimicrobial and antiviral activities. Karpova et al. proved the antifungal activity of the dry biomass of *Penicillium chrysogenum* F-24-28 along with its combination with azoxystrobin for efficient crop protection [4]. According to in vitro experiments, the supplementation of an agarized medium with *P. chrysogenum* F-24-28 strain DMP (7.5–10 g/L) resulted in a significant growth inhibition in several plant-pathogenic *Fusarium* fungi. The combination of DMP with a commercial azoxystrobin-based fungicide resulted in a prolonged growth inhibition in *F. oxysporum*, *F. graminearum* and *F. culmorum*, even at fungicide concentrations significantly below the recommended level (0.5–2.5 mg/L or 2.5–12.5 g/ha vs. the recommended 100–275 g/ha). These results demonstrate that the *P. chrysogenum* F-24-28 strain DMP biopreparation might be suitable

to control crop diseases caused by a wide range of plant pathogens, and to prevent the possible selection and spreading of resistant pathogen strains.

A common problem with previous research carried out on biopesticides is that data are generated in the laboratory and only seldom tested under field or greenhouse conditions. However, this change in scale might have tremendous effects on efficacy. In this context, Dunan et al., in a series of experiments on an increasing scale, reported that botanical pesticides based on essential oils (EOs) could be promising alternatives to combat aphids [5]. In particular, the entomotoxicity of green anise and fennel EO fumigation was tested on the potato aphid, *Macrosiphum euphorbiae*. Three different settings of increasing scales were considered (leaflet, whole plant and greenhouse) to appraise the consistency of EO impact from controlled laboratory to greenhouse production conditions. LC₅₀ values for green anise and fennel were 6.6 µL L⁻¹ air and 12.2 µL L⁻¹ air, respectively, based on dose–response curves in leaflet experiments, but fennel EO induced phytotoxicity. EO efficiency was confirmed at the whole-plant scale. In the greenhouse experiment, fennel EO exhibited a greater efficiency than at the laboratory scale, equaling green anise EO efficiency but both EOs showed delayed phytotoxicity, illustrating the importance of long-term monitoring. The present study revealed the ability of both EOs to control *M. euphorbiae* populations under greenhouse conditions and hinted at the importance of assessing EO efficiency in realistic agronomic conditions (e.g., under the fluctuating environmental conditions usually occurring in greenhouses).

Finally, Nikolaou et al. reported a bibliographic growing interest in biocidals based on plant secondary metabolites, but there has been little authorization implemented by the European Council for the protection of stored grains [6]. According to the authors, resistance issues and ecotoxicity concerns necessitate the development of ecofriendly tools in this context. In their review, they refer to the recent findings on plant extracts and pure plant-derived substances with promising biological activities and the potential to be used as biopesticides for stored products. The main aim of biopesticides is to be effective against target pests without harming humans and the environment. Many plant species, among those reported in the study, are part of the human diet, and thus are not harmful to humans. Edible plant extracts produced with inorganic solvents represent safe candidates for use as repellants, fumigants or contact pesticides. Cinnamon, rosemary, parsley, garlic, oregano and basil are found in products intended for human consumption but also display significant biological activities. Interestingly, cinnamon is one of the most widely tested botanical matrixes, exhibiting the most lethal effects on almost all insect and mite taxa reported in the study (Acaroidea, Coleoptera and Lepidoptera), followed by basil and garlic. *Prunus persica*, *Azadirachta indica* A. Juss and *Carum* sp. appear to also be very promising as miticides and/or insecticides, with *A. indica* already being represented commercially by a plant-derived acaricidal formulation.

In summary, natural substances with significant biological activity are appropriate for use as pesticides and biocides. They also exhibit secondary beneficial attributes, such as resistance management, and work synergically with commercial pesticides and soil microcosms' enhancement properties. Assessing their efficacy under real field conditions is essential for efficiency verification.

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

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