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Posted Date: 18 December 2024

doi: 10.20944/preprints202412.1463.v1

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Article

Evaluation of Pinocembrin Dihydrochalcone Properties for Potential Use as a Biopesticide

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Abstract: The excessive use of synthetic pesticides has not only resulted in increased resistance among weeds and pests, leading to significant economic loss but has also raised serious health and environmental concerns. In line with the European Green Deal 2030, and its Farm to Fork strategy, a 50% reduction of chemical pesticide is aimed by 2030, promoting a transition towards more sustainable, natural alternatives. Chalcones and their derivatives, known for their herbicidal, fungicidal, bactericidal, and antiviral properties, are emerging as promising candidates. These naturally occurring compounds have long been recognized for their beneficial health effects and wide-ranging applications. Dihydrochalcones, a class of secondary metabolites, continue to attract growing interest in both biological and pharmacological fields due to their health-promoting properties, making them potential candidates for further research and development. However, their limited concentration in plants, along with poor solubility and bioavailability, brings challenges for their development. The aim of this study was to provide information on the properties of synthetic substance, namely pinocembrin dihydrochalcone (3-phenyl-1-(2,4,6-trihydroxyphenyl)-1-propanone) such as soil dissipation and adsorption in the soil and its potential for degradation. Additionally, antioxidant activity was assessed using DPPH assay along with reducing power experiments.

Keywords: pinocembrin dihydrochalcone; biopesticide; soil dissipation; partition coefficient; DPPH

1. Introduction

Biopesticides, which are safer alternatives to conventional pest control chemicals, are primarily derived from natural sources. These include microbial and biochemical biopesticides, as well as plant-incorporated protectants (PIPs). Together, they currently account for 5% of the global pesticide market [1,2]. Chalcones and their derivatives have garnered significant attention due to their wide range of nutritional and biological activities, such as anti-inflammatory, antitumor, antibacterial, antifungal, antimalarial, antitubercular, and anti-pigmentation effects, often demonstrating remarkable efficacy [3]. They are also recognized for their role as bioprotectants [4,5]. Dihydrochalcones, naturally biosynthesized through the well-understood phenylpropanoid pathway in plants, were initially believed to be limited to around 30 plant families. However, along with more advanced analytical techniques development, it has been discovered that

dihydrochalcones are more widely distributed in nature. Apples are considered as the richest dietary source of these compounds, which are also found in leaves and roots of apple and hairy roots obtained through genetic transformation [6]. In addition, dihydrochalcones were also found in other different plant families, including *Asteraceae*, *Lauraceae*, and *Salicaceae* [7,8].

Dihydrochalcones have demonstrated effectiveness against a wide range of target pathogens, including fungi such as *Phytophthora*, *Pythium*, downy mildew (*Pseudoperonospora cubensis*, *Plasmopara*), *Colletotrichum*, *Rhizoctonia*, and rust (*Puccinia triticina*), as well as bacteria like *Xanthomonas* and *Pseudomonas*. These compounds have been tested on various host plants, including vegetables, leafy greens, fruits, and wheat [9,10].

Werner et al. (2020) demonstrated that 2',4'-dihydroxyhydrochalcone and its synthetic analogs show great promise as potential anti-*Saprolegnia* agents, largely due to their high lipophilicity, particularly in molecules with alkyl chains longer than 10 carbon atoms. These compounds are considered promising scaffolds for the development of new, potent anti-oomycete agents [11].

Antimicrobial activities very often go hand in hand with antioxidant properties. To assess these properties, spectrometric antioxidant tests, such as DPPH and reducing power assays are commonly employed.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is a widely used, quick, simple, and cost-effective method for evaluating antioxidant activity by using free radicals. This assay measures the ability of compounds to act as hydrogen donors or free radical scavengers (FRS). In the DPPH test, a stable free radical, DPPH, is reduced in the presence of an antioxidant, leading to a change in its distinctive purple color, which has a strong absorbance peak at 517 nm. When an FRS antioxidant reacts with DPPH, it forms DPPH-H, resulting in decreased absorbance and a color shift from purple to yellow as hydrogen atoms are donated. This color change indicates a reduction in radical concentration, directly reflecting the antioxidant's capacity to neutralize free radicals. As the DPPH solution interacts with a hydrogen-donating compound, it transforms into diphenylpicrylhydrazine, losing its violet color, which signifies the antioxidant's electron-donating power [12,13]. The reducing power is determined by the reduction of Fe (III) to Fe (II) in the presence of the tested compound. The concentration of Fe (II) can be monitored by measuring the formation of Prussian blue at 700 nm. The higher dye concentration and therefore the higher absorbance, indicates greater reducing power and enhanced antioxidant activity of the tested compound.

Information on the degradability of organic chemicals is valuable for both hazard and risk assessments. Aquatic hazard classification and general hazard assessments typically rely on standardized tests for ready biodegradability. However, data from tests simulating biodegradation in environments such as water, aquatic sediment, and soil can also contribute to these samples' assessment [14]. The European Commission's Technical Guidance Document on Risk Assessment suggests that for readily biodegradable chemicals meeting the pass criteria and the 10-day window, a degradation rate constant (k) of 1.0 hour^{-1} , with a half-life of 0.69 hours, can be used in models estimating chemical removal in sewage treatment plants (STP models). If a chemical passes the level within the 28-day test period but fails the 10-day window, a lower rate constant of 0.3 hour^{-1} , corresponding to a half-life of 2.3 hours, may be applied in STP models [15].

While the antimicrobial activities and effectiveness of biopesticides are crucial, their safety for humans and the environment is equally important, encompassing factors such as toxicity, persistence, and other related concerns. The persistence and dissipation of active substances are measured using DT (dissipation time), especially DT_{50} and DT_{90} values, which represent the time required for 50% and 90% of a substance, respectively, to disappear. "Disappearance" encompasses all processes leading to the substance's transformation, degradation, and eventual mineralization, including microbial degradation, chemical hydrolysis, photochemical reactions, and other mechanisms like leaching, volatilization, and plant uptake. In soil, laboratory experiments generally consider a DT_{50} of less than 60 days at 20°C acceptable (or less than 90 days at 10°C for applications in colder regions). In field conditions, acceptable thresholds are a DT_{50} of less than 3 months and a DT_{90} of less than 1 year. It should be noted that DT_{50} and DT_{90} indicators do not differentiate between degradation processes—such as mineralization, microbial degradation, hydrolysis, or photochemical

reactions—and loss through leaching, volatilization, or plant uptake. Instead, these values provide a holistic assessment of a substance's dissipation, encompassing all contributing processes [16]. In terms of adsorption - it depends on two main factors - pesticide properties and soil properties showing the behavior of pesticide in soil. Molecular properties of pesticides like solubility, ionisability and hydrophilicity and lipophilicity are crucial [17].

Determination of antioxidant activity in the case of biopesticides is important in the context of assessing their biodegradability in the environment, because antioxidants are able to influence the decomposition processes associated with radicals and redox reactions [18].

Various analytical methods are available for determining chalcone concentrations across different matrices, including chromatography with a range of detectors, MALDI, and HNMR techniques [3]. Pompermaier et al. employed an UHPLC-DAD method to identify six primary dihydrochalcone derivatives [19]. In this experiment, UHPLC-ESI-MS/MS was used to determine the DT_{50} and DT_{90} values as well as to assess the dihydrochalcone concentration after sorption experiment.

The aim of the study was to evaluate the attributes of pinocembrin dihydrochalcone, including its antioxidant properties and environment safety as potential biopesticide. This could be assessed through the soil dissipation study experiment as well as organic carbon partition coefficient, K_{oc} .

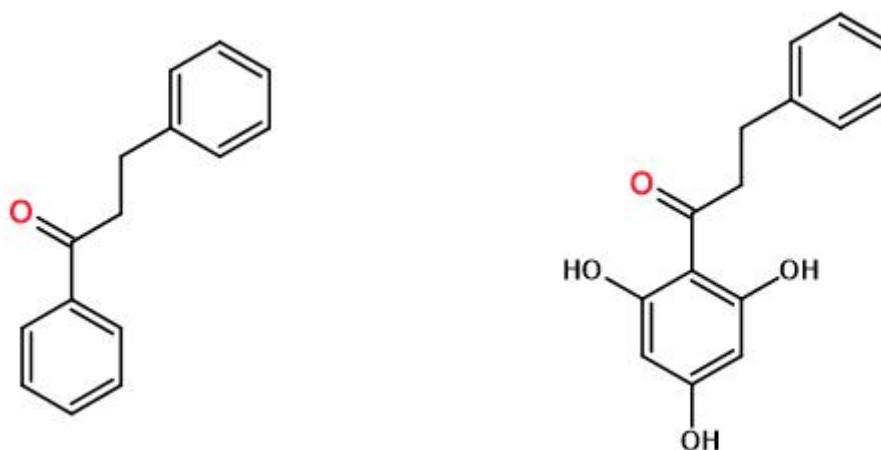


Figure 1. Dihydrochalcone (left) and pinocembrin dihydrochalcone (3-phenyl-1-(2,4,6-trihydroxyphenyl)-1-propanone) (right).

2. Results

Dissipation Study Results

The dissipation study aimed to determine the DT_{50} (time for concentration to reduce to 50% of its initial value) and DT_{90} (time for concentration to reduce to 10% of its initial value) for pinocembrin dihydrochalcone. Results obtained in two different soils: Biostrateg and Laskowice, indicated that dissipation of dihydrochalcone follows single first-order (SFO) kinetic model, with very rapid degradation. For Biostage model DT_{50} and DT_{90} reached 1.3 and 5.46 days, respectively, while in the case of Laskowice soil relevant values were 0.75 and 4.55 days. [Figure 2 and Figure 3].

These findings align with those of Pedrinho et al. who reported a dihydrochalcone dissipation rate of 2.3 to 9.85 days (for DT_{50} and DT_{90}) across three soil types, also described by the SFO model [9].

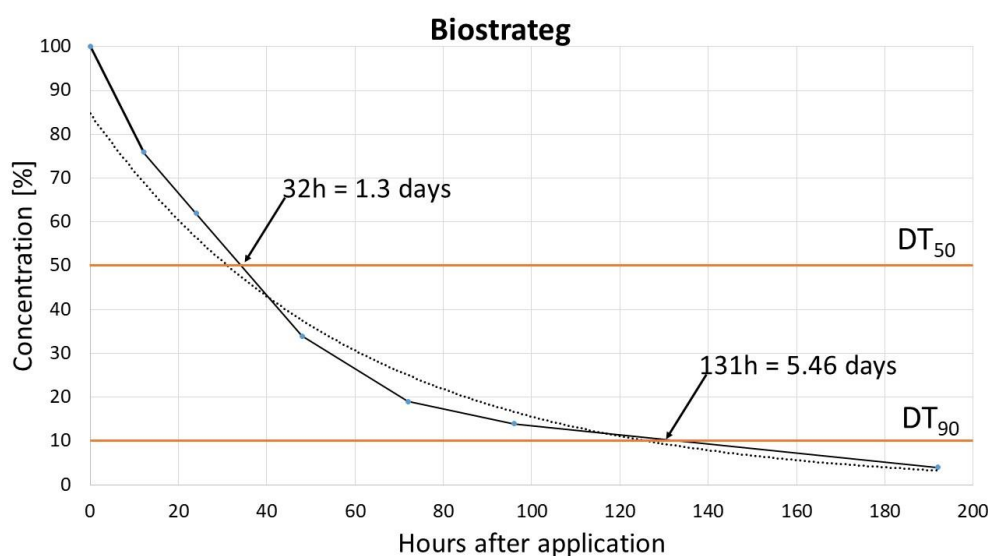


Figure 2. Biostrateg soil dissipation model.

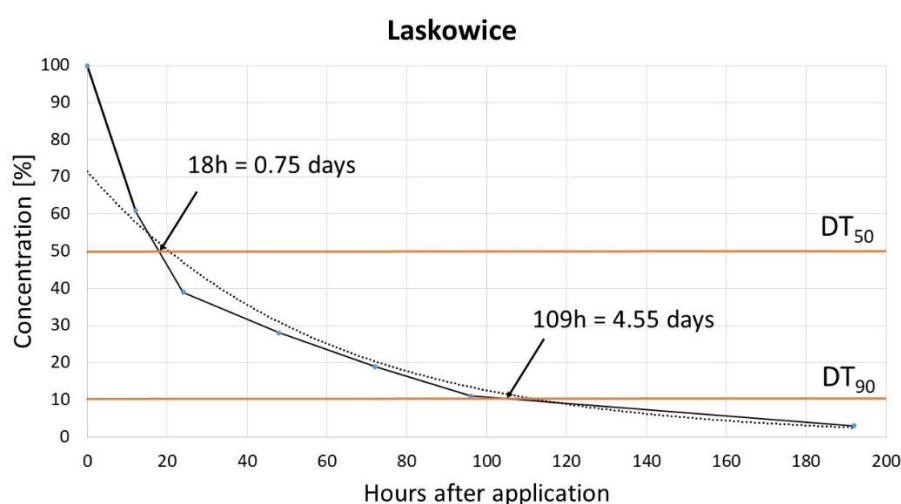


Figure 3. Laskowice soil dissipation model.

Partition Coefficient

Accumulation of pesticides belonging to organic compounds in soils is the result of complex processes such as partitioning, sorption, desorption and diffusion, which involve both the mineral and organic fractions of the soil. These processes determine the persistence and residence of these compounds in the soil as well as their potential for degradation. Partition coefficients- K_F are used to describe the binding capacity and release of organic compounds in soil [20]. In our study, the sorption of pinocembrin dihydrochalcone exhibited the character of a Freundlich curve model which indicates the nonlinear nature of the binding processes in the soil, involving both absorption and adsorption [Figure 4]. This model suggests that contaminant molecules are influenced by active sites (functional groups on soil components) that vary in both quantity and quality, which in turn affects the strength of pinocembrin dihydrochalcone adsorption to the soil.

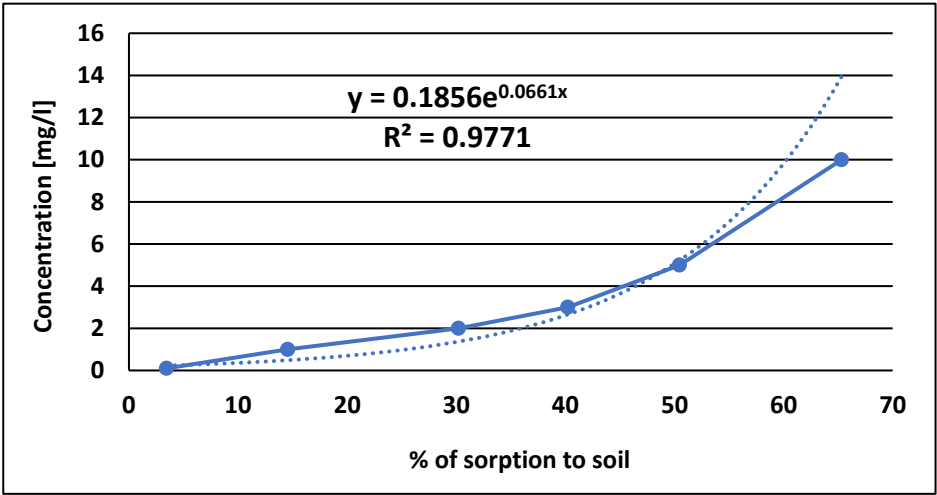


Figure 4. Fitting of sorption model of dihydrochalcone to soil.

Pesticides with a high value of the K_F coefficient typically exhibit a pronounced tendency to sorb in the soil and a limited desorption capacity. However, in our study the K_F value was measured at 0.0661 which indicates its low affinity for soil and resistance to aging processes. The result of partitioning is sorption, or the process, of binding pesticides to the solid phase. Observed sorption processes in studied soil occur probably between the soil solution in which these compounds are dissolved and organic/mineral matter. This is due to the high affinity of these compounds mainly to nonpolar soil components characterized by a high carbon-to-oxygen ratio – such as organic matter [21,22]. The binding of pesticides can take place inside the three-dimensional structures of organic matter (absorption) and/or on the surface of various components as a result of physical (physical adsorption) and chemical (chemical adsorption) processes. The rate of these sorption processes occurs mainly limited by the rate of diffusion of contaminants in the soil matrix [23].

DPPH Results

Anti-radical activity (DPPH scavenging) was observed in the water solution, within the tested concentrations. The scavenging effect was the strongest at a concentration of 0.1 mg/mL, reaching a plateau after 1.5 hours. As the concentration decreased, the time required to achieve peak activity increased correspondingly. Referring to obtained results the half maximal effective concentration (EC50) of pinocembrin dihydrochalcone was calculated to be 0.0096 mg/mL. Within the typically used range in agricultural applications (300-600 ppm), the radical scavenging activity did not reach the effective concentration [Figure 5].

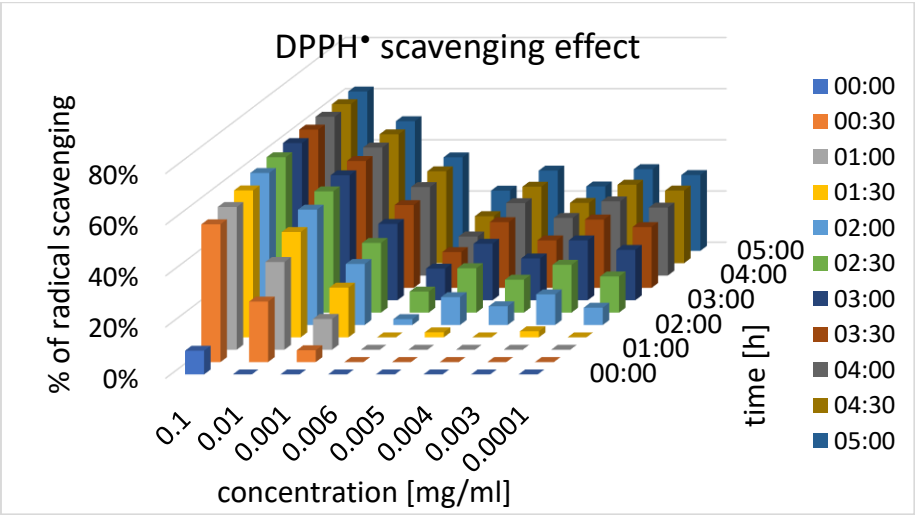
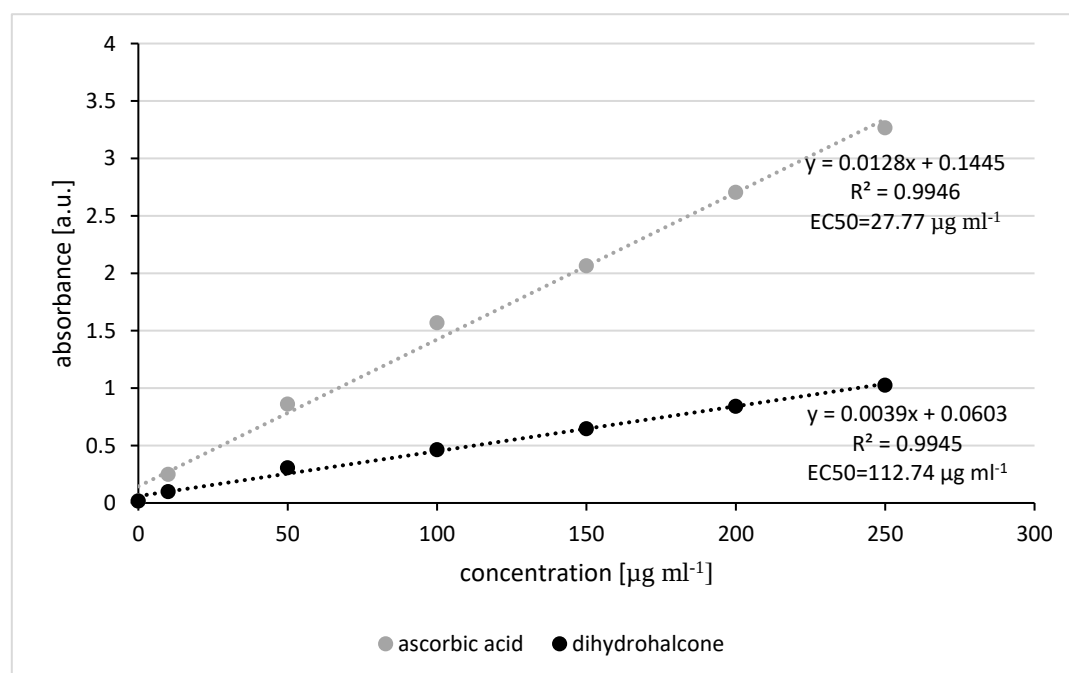


Figure 5. DPPH scavenging effect.*Reducing Power*

The reducing power of dihydrochalcone was lower than that of ascorbic acid, which serves as the reference sample. The EC_{50} values for dihydrochalcone and ascorbic acid were 112.74 and 27.77 $\mu\text{g mL}^{-1}$, respectively [Figure 6]. Antioxidant activity increased proportionally with concentration within the tested range. These findings align with similar studies on the reducing power of various dihydrochalcones. For instance, Chen et al. (2017) [24] reported that phloridzin, a dihydrochalcone, and the glycoside of phloretin displayed reducing power comparable to ascorbic acid in the concentration range of 100-300 $\mu\text{g mL}^{-1}$. In case of pinocembrin dihydrochalcone differences were observed in a concentration dependent manner.

**Figure 6.** The reducing power of dihydrochalcone in comparison with ascorbic acid.**3. Discussion**

The antioxidant activity of dihydrochalcone was assessed through DPPH radical scavenging, and the results revealed a dose-dependent response. In the water solution, the scavenging effect was most pronounced at a concentration of 0.1 mg/mL, reaching a plateau after 1.5 hours. As the concentration of dihydrochalcone decreased, the time required to achieve peak activity increased proportionally, indicating that higher concentrations lead to quicker onset of antioxidant activity. This behavior suggests that dihydrochalcone has the potential for effective antioxidant action, though its effectiveness in agricultural applications may be limited by the concentration range typically used in such contexts. Specifically, in the 300-600 ppm range, common in agricultural applications, the scavenging activity did not reach the effective concentration (EC_{50}), which was calculated to be 0.0096 mg/mL. These findings suggest that dihydrochalcone may be more effective as an antioxidant at higher concentrations than those typically applied in agriculture. The concentration is just as important as the structure of the dihydrochalcone, where the number of phenolic hydroxyl groups may enhance antioxidant activity, while glycosylation could reduce it [25,26].

The reducing power of pinocembrin dihydrochalcone was found to be lower than that of ascorbic acid, with EC_{50} values of 112.74 $\mu\text{g/mL}$ for pinocembrin dihydrochalcone and 27.77 $\mu\text{g/mL}$ for ascorbic acid. This indicates that while pinocembrin dihydrochalcone has antioxidant potential, it is less potent than ascorbic acid in terms of reducing power. However, the observed linear increase in antioxidant activity with concentration suggests that pinocembrin dihydrochalcone could still be

a valuable antioxidant at appropriate concentrations. These findings are consistent with previous studies, such as those by Chen et al. (2017) [24], who reported comparable reducing power between phloridzin (a dihydrochalcone) and ascorbic acid within a concentration range of 100-300 µg/mL.

The dissipation study, which aimed to determine the degradation rates of pinocembrin dihydrochalcone, revealed that it follows a single first-order (SFO) kinetic model. Rapid degradation of pinocembrin dihydrochalcone was observed, with a DT₅₀ (time for concentration to decrease to 50% of its initial value) ranging from 0.85 to 1.7 days and a DT₉₀ (time for concentration to decrease to 10%) between 4.55 and 5.26 days. These findings align with the results reported by Pedrinho et al. [9], who also observed rapid dissipation of dihydrochalcone across different soil types, suggesting that dihydrochalcone breaks down quickly in the environment, reducing its persistence and potential for accumulation. This rapid degradation may be advantageous for minimizing environmental impact, but it also implies that repeated or continuous applications may be necessary for sustained effectiveness in agricultural settings. Soil degradation rates can be influenced by various factors, including the soil's physicochemical properties (such as pH and organic carbon content), biological properties (such as microbial activity and distribution), and environmental conditions, like temperature and moisture. The soil chemical properties also play a role in determining both the degradation route and rate. Degradation rates may also vary, with multiple studies confirming significant field-to-field differences in pesticide dissipation [26,27].

The sorption characteristics of pinocembrin dihydrochalcone were also studied, and the results indicated that its sorption to soil followed a Freundlich isotherm, which is indicative of a nonlinear adsorption process involving both absorption and adsorption mechanisms. The Freundlich model suggests that pinocembrin dihydrochalcone interacts with soil through active sites that vary in both quantity and quality, influencing the strength of adsorption. The measured partition coefficient (KF) value of 0.0661 suggests a low affinity for soil, indicating that pinocembrin dihydrochalcone is less likely to be strongly bound to soil particles. This result implies that pinocembrin dihydrochalcone may be subject to quicker leaching from the soil, reducing its potential for long-term soil residence and effectiveness. Furthermore, the low KF value suggests that pinocembrin dihydrochalcone is less likely to undergo aging processes that would otherwise increase its persistence in the soil.

Overall, the findings of this study highlight the promising antioxidant potential of dihydrochalcone, particularly in water solutions, while also underscoring the influence of environmental factors such as solvent type, concentration, and soil interactions on its bioactivity. While pinocembrin dihydrochalcone exhibits a moderate antioxidant effect and rapid degradation in the soil, its low affinity for soil and rapid dissipation suggest that it may be more effective in short-term applications, with careful consideration needed for its persistence and long-term efficacy in agricultural settings.

4. Materials and Methods

Material

The tested material, pinocembrin dihydrochalcone, was supplied by Methabolic Insight Ltd. (Ness Ziona, Israel). Pinocembrin dihydrochalcone (3-phenyl-1-(2,4,6-trihydroxyphenyl)-1-propanone) is a white, amorphous powder, produced synthetically with 99,5% purity.

Soil Dissipation Studies

The dissipation study of dihydrochalcone was done under controlled laboratory conditions using growth chambers. Field studies are only required when the DT₅₀, determined under laboratory conditions at 20°C and a soil moisture content corresponding to a pF value of -10 to -32 kPa, exceeds 60 days.

The experiment followed the OECD 307 protocol with slight modifications [28]. Soil samples were collected from the upper layer (0-15 cm) of two soil types, Laskowice and Biostrateg, both free of dihydrochalcone residues. After passing the soil through a 2-mm sieve, it was stored in covered trays in a greenhouse for 10 days with regular mixing. Soil moisture content was measured by drying

samples at 105°C for 24 hours and calculating the weight difference. To standardize conditions, soil moisture was maintained at 60% of field capacity, monitored regularly, and adjusted with distilled water as needed.

Soil samples were placed in 60 mm diameter pots (height: 55 mm), with three replicates per variant. These pots were then transferred to growth chambers set to a day/night temperature cycle of 20°C, with light intensity at $250 \pm 10 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux. Two days after setting up the pots in the chambers, dihydrochalcone was applied using a stationary chamber sprayer equipped with a TeeJet XR 11003-VS mobile nozzle. The application rate matched field conditions (200 L/ha). Soil samples (one pot, approximately 150 g, per sample/replicate) were collected at 1 hour (initial concentration) and at 1, 3, 6, 12, 21, and 36 days after treatment (DAT).

For analysis, soil samples were extracted with methanol using a high-pressure, high-temperature extractor. The methanolic extract was then centrifuged at 7500 rpm, and the supernatant was used. The quantitative analysis of dihydrochalcones was performed using ultrafast liquid chromatography coupled with a triple quadrupole mass spectrometer (UHPLC-MS/MS).

Chamber Conditions:

Day period: 6 hours with 10% light intensity.

Night period: 16 hours with no light.

Basic Physicochemical Properties of the Soil

Key physicochemical parameters of the two soil types - Laskowice and Biostrateg - were analyzed, including pH, phosphorus, and potassium content, as well as granulometric composition. Soil pH was measured potentiometrically using a 1 M KCl solution, and phosphorus and potassium content was determined using the Egner-Riehm method. Soil texture analysis was performed with the modified Casagrande method [29,30].

Soil properties by type:

Laskowice Soil:

pH: 6.1

Texture: Sand 78%, Silt 22%, Clay 0%

Phosphorus (P_2O_5): 14 mg/100 g soil

Potassium (K_2O): 19 mg/100 g soil

Soil moisture: 15.98%

Nitrogen (N): 72-82 kg/ha

Biostrateg Soil:

pH: 7.04

Texture: Sand 22%, Silt 34%, Clay 41%

Phosphorus (P_2O_5): 21.3 mg/100 g soil

Potassium (K_2O): 18.9 mg/100 g soil

Soil moisture: 15.99%

Nitrogen (N): 71-90 kg/ha

Identification and Quantification of Pinocembrin Dihydrochalcone

The identification and quantification of pinocembrin dihydrochalcone after soil dissipation studies and partition coefficient experiment was conducted using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) with a triple quadrupole analyzer. Chromatographic separation was achieved using a Shimadzu Prominence UFLC system (Shimadzu, Kyoto, Japan), equipped with an LC-30ADXR binary solvent manager, DGU-20A3 degasser, CTO-10ASVP column oven, Kinetex C18 column (2.6 μm particle size, 50×3.0 mm, Phenomenex, Torrance, CA, USA), SIL-20AXR autosampler, and CBM-20A system controller. Separation was performed under reversed-phase conditions, with a mobile phase of water (A) and acetonitrile (B) in a binary gradient, containing 32% water. The total analysis time was 10 minutes, with a flow rate of 0.40 mL/min, a column temperature of 35°C, and a sample injection volume of 10 μL .

For dihydrochalcone analysis, the LCMS-8030 tandem mass spectrometer (Shimadzu, Kyoto, Japan) with a triple quadrupole analyzer and an electrospray ionization (ESI) source operating in negative ionization mode was employed. Nitrogen, used as both drying and nebulizing gas, was supplied from pressurized air, with flow rates of 15 L/min and 3 L/min, respectively. The desolvation line was maintained at 250°C, and the heat block at 400°C. Collision-induced dissociation (CID) utilized high-purity argon (99.99%) at a pressure of 230 kPa, with a dwell time of 10 ms. Quantitative data were processed using LabSolution Ver. 5.6 (Shimadzu, Kyoto, Japan) software.

Pinocembrin dihydrochalcone identification and quantification was conducted in multiple reaction monitoring (MRM) mode. The dihydrochalcone peak appeared at 0.65 minutes, with an RSD% for retention time below 2%. Negative ionization mode was used for the analysis, with the following transitions selected for dihydrochalcone identification and quantification:

MRM 1: 257.26 > 213.15, dwell time 100 ms, collision energy (CE) = 19

MRM 2: 257.26 > 81.00, dwell time 100 ms, CE = 29

MRM 3: 257.26 > 173, dwell time 100 ms, CE = 35

The optimized method is highly specific and robust. Despite the rapid dissipation of pinocembrin dihydrochalcone, the analyte remained detectable throughout the experiment.

Partition Coefficient

The partition coefficient of dihydrochalcone was determined experimentally based on its sorption into the soil. A silt loam soil, representative of agriculturally used soils in Poland, was selected for the study. The soil composition included 35% sand, 63% silt, and 2% clay, with the following properties: pH(KCl) = 7.4, total carbon (TC) = 9.02 g kg⁻¹, total nitrogen (TN) = 0.97 g kg⁻¹, and total organic carbon (TOC) = 7.74 g kg⁻¹. It was free from contamination by polycyclic aromatic hydrocarbons, pesticides, and heavy metals, ensuring reliable results by minimizing interference with the sorption process. The distribution coefficient (K_F) was determined by plotting a logarithmic curve fitted to the experimental data. Sorption studies were conducted using test solutions with varying dihydrochalcone concentrations (0.1 mg kg⁻¹, 1 mg kg⁻¹, 2 mg kg⁻¹, 3 mg kg⁻¹, 5 mg kg⁻¹, and 10 mg kg⁻¹), with each concentration measured in duplicate. The relative standard deviation (RSD) was < 2%. A matrix blank and a standard were included in the analysis to assess compound stability during the sorption period. Sorption was carried out over 24h at a constant temperature of 20°C, with 5 ml of the test solution and 1 g of soil shaken on a rotary shaker at 120 rpm. The concentration of dihydrochalcone in the solution was determined using LC-ESI-MS/MS method described above.

DPPH Assay

The DPPH radical scavenging assay was conducted using a modified version of the method developed by Brand-Williams et al. (1995). Pinocembrin dihydrochalcone was initially dissolved in methanol at a concentration of 1.0 mg/mL, then further diluted in water to final concentrations of 0.1, 0.01, 0.001, 0.006, 0.005, 0.004, 0.003, and 0.0001 mg/mL. Each concentration, was transferred to a 96-well plate in eight replicates. An equal volume of 0.2 mM methanolic DPPH• solution was added to each well. Absorbance was measured immediately at 517 nm using a BioTeq µQuant™ Microplate Spectrophotometer, and then recorded every 30 minutes over a 5-hour period during which samples were incubated in the dark at room temperature. Pure water without tested compound mixed with DPPH• solution was used as a control.

The scavenging effect percentage was calculated with the following formula:

$$\% \text{ scavenging} = \frac{Abs_{DPPH} - (Abs_{sample} - Abs_{control})}{Abs_{DPPH}} \times 100$$

where:

Abs_{DPPH} is the absorbance of DPPH• in water

Abs_{sample} is the absorbance of the sample with DPPH•

Abs_{control} is the absorbance of the sample without DPPH•

From the obtained results, the EC50 value for the water-based solution was calculated using AAT Bioquest, Inc. (2024) [31].

Reducing Power

The antioxidant activity of dihydrochalcone was further evaluated through its reducing power following a method described by Oyaizu (1986) [32] and then slightly modified by Oleszek and Kozachok (2018) [33]. Briefly, 1 ml aliquots of dihydrochalcone, with concentrations ranging from 0 to 250 $\mu\text{g ml}^{-1}$, were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium (III) ferricyanide. The mixture was incubated at 50°C for 30 min. Next, 2.5 ml of 10% trichloroacetic acid (TCA) was added, and the samples were centrifuged at 6000 rpm for 10 min. The resulting supernatant (2.5 ml) was diluted with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric (III) chloride. The absorbance was measured at 700 nm. The results were expressed as the effective concentration (EC_{50}), which represents the concentration corresponding to an absorbance of 0.5. Ascorbic acid, strong antioxidant, was used as reference sample.

5. Conclusions

The DPPH assay demonstrated that pinocembrin dihydrochalcone exhibits moderate anti-radical (scavenging) activity. The highest radical scavenging activity occurred at a concentration of 0.1 mg/mL, stabilizing after 1.5 hours. Lower concentrations required more time to reach maximum activity, indicating a concentration-dependent scavenging effect. However, in the 300–600 ppm range typical for agricultural applications, the scavenging effect was insufficient to reach the effective concentration (EC_{50}), which was calculated at 0.0096 mg/mL. This suggests that the radical scavenging potential of dihydrochalcone may be limited under practical agricultural conditions, especially at lower concentrations. Furthermore, the dissipation study showed that dihydrochalcone degrades rapidly in soil, following a single first-order (SFO) kinetic model. The DT_{50} and DT_{90} values fell within a very short range, indicating rapid breakdown of the compound in soil environments. This rapid dissipation suggests that pinocembrin dihydrochalcone has a limited persistence in soil, which may reduce environmental impact but also affect its potential as a long-term agricultural additive. The biodegradation in soils involves free radicals, so the questions arise about the function of antioxidant properties in such degradation. Polyphenolic compounds with known antioxidant properties are rather considered as relatively resistant to degradation, thanks to its antiradical activity. Nonetheless, the results of present studies proved that the degradation of pinocembrin dihydroflavone is fast.

Together, the findings imply that while dihydrochalcone poses moderate radical scavenging activity, it may not be effective at typical agricultural concentrations. Its rapid dissipation in soil further suggests limited longevity, which could reduce its efficacy over time. Future research might explore methods to enhance its stability and activity in both soil and agricultural settings.

Author Contributions: “Conceptualization, M.D.-B.; methodology, M.D.-B, M.O., A.U.-J., W.K.; software, M.D.-B., M.K.; validation, M.D.-B. and W.K.; formal analysis, M.D.-B, M.O., W.K. ; investigation, M.D.-B.; data curation, M.D.-B., M.K., W.K., S.Z.; writing—original draft preparation, M.D.-B, M.O., A.U.-J., S.Z. ; writing—review and editing, M.D.-B., S.Z., B.N.; visualization, M.D.-B.; supervision, M.D.-B. and S.Z. ; project administration, M.D.-B.; funding acquisition, M.D.-B., M.O., M.K. All authors have read and agreed to the published version of the manuscript.” Please turn to the [CRediT taxonomy](#) for the term explanation. Authorship must be limited to those who have contributed substantially to the work reported.

Funding: This research is supported by the European Union’s Horizon Europe research and innovation programme under the grant agreement No 101084163 project RATION.

Institutional Review Board Statement: “Not applicable”.

Informed Consent Statement: “Not applicable.”.

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still

required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at <https://www.mdpi.com/ethics>.

Conflicts of Interest: “The authors declare no conflicts of interest.”

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