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Evaluation of *Magonia pubescens* A. St.-Hill. Roots Extract against Phytopathogens: Searching for Eco-Friendly Crop Protection Products

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Abstract: Botanical biopesticides have emerged as sustainable and eco-friendly alternatives to synthetic pesticides, whose indiscriminate use leads to several drawbacks to human and environmental health. To the best of our knowledge, there have been no reports on *M. pubescens'* bioactivity on phytopathogens affecting crops as a potential fungicide or antifeedant. This has encouraged us to investigate the potential of the roots of this plant as a source of biopesticides. The present study reports on the evaluation of the roots extract from *Magonia pubescens* A. St.-Hill., a species from the Cerrado (Brazilian savannah), on the phytopathogenic fungi *Botrytis cinerea*, *Fusarium oxysporum*, and *Alternaria alternata*. In addition, its insect antifeedant effect was assayed against *Chrysodeixis chalcites*. Thus, an in vitro test-assay was used to determine the fungicide potential (percentage growth inhibition, % GI) of the ethanolic extract of this plant species, whereas a leaf-disk bioassay on the 5th instar larvae of *C. chalcites* was performed to evaluate its insecticidal potential. The ethanolic extract was further fractionated by liquid–liquid partition using solvents of increasing polarity. The hexane/dichloromethane fraction exhibited a moderated potency and was similar to the ethanolic extract on the three assayed fungi (around % GI 30 at 1 mg/mL), whereas the *n*-butanol fraction showed a slight improvement of the fungicide effect against *B. cinerea* (% GI 39.18 at 1 mg/mL). Moreover, the ethanolic extract exhibited a strong antifeedant activity, with a refusal rate (FR) higher than 90% in both choice and non-choice assays against *C. chalcites*, while the ethyl acetate and *n*-butanol fractions behaved as appetite suppressors. These results highlight *M. pubescens* as a promising source of biopesticides and deserve further investigations to optimize extraction procedures.

Keywords: *Magonia pubescens*; biopesticides; phytopathogenic fungi; *Chrysodeixis chalcites*

1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), it is estimated that the world population will increase by over 32% by 2050, which will mean an increase in the demand for food [1]. This fact highlights the need to adopt strategies that ensure the efficiency, productivity, sustainability, and security of our food supply. One of the challenges to achieve these goals is the control of pests affecting crops, which, in recent times, have increased due to globalization, climate change, and the emergence of resistance to conventional pesticides [2,3]. Among the pests that most severely affect crops, fungal infections via ascomycetes, such as *Botrytis cinerea* [4], *Fusarium oxysporum* [5], and *Alternaria*

alternata [6], have a great impact worldwide. In Brazil, *B. cinerea* affects several crops, mainly eucalyptus in the southwest region [7], and grapes [8] and strawberries [9] in the south. Recent studies show that some strains of this fungus are resistant to some commonly used fungicides such as external quinone inhibitors (QoI) [10]. Moreover, *F. oxysporum* causes banana Fusarium wilt, a pandemic that has threatened crops and the export of this fruit in Brazil, which is the fourth largest banana producer in the world [11,12]. Regarding Alternaria brown spot caused by *A. alternata*, this affects mainly tangerine crops, in which Brazil is the fifth largest producer [13]. Moreover, there are few fungicides registered in the country that are able to control the spread of this fungus, and there is evidence of the emergence of resistant strains of this pathogen [10].

Furthermore, the golden twin-spot moth, *Chrysodeixis chalcites* (Esper) (Lepidoptera: Noctuidae), is a highly polyphagous species that causes severe damage to crops in many regions of Europe, the Mediterranean, the Middle East, and Africa [14]. The larvae of this moth feed on several crops, such as bananas, cabbages, beans, corn, potatoes, or tomatoes [15]. Moreover, *Chrysodeixis includens* is a pest that affects crops in North and South America, especially in the USA, Argentina, and Brazil. In Brazil, the insect is one of the major defoliators of soybean crops, especially relevant as the country is the world's leading soybean producer and exporter [16].

Currently, crop protection relies almost exclusively on the use of synthetic pesticides [3]. However, their indiscriminate use [17] has aroused social concerns due to overwhelming evidence of their detrimental effects on the environment and human health, increasing the risks for the development of resistant strains and the erratic supply of beneficial organisms [18]. Therefore, there is a need for research on eco-friendly pesticides for their use in agricultural pest management programs. Botanical biopesticides are considered as safe, ecologically sound, and sustainable alternatives to synthetic pesticides [19,20]. They have key advantages over traditional pesticides, including lower toxicity, they are easily biodegradable, they exclusively affect the pest in question and closely related species, they present new modes of action, and can be used in organic agriculture [21–23]. In fact, several plants and plant-derived products have been reported as botanical biopesticides [24,25], and pure natural products such as Azadirachtin and pyrethrins are the basis of most current, commercial botanical insecticides [19].

The sapindaceae family comprises about 140 genera and 1900 species, distributed mainly in tropical regions of the world. In Brazil, the family is represented by 28 genera and 417 species [26]. *Magonia pubescens* A. St.-Hil. (Sapindaceae) is an endemic species from Brazil popularly known as 'tingui' or 'timbó'. It is the only species of the *Magonia* genus and is widely distributed in the states of Goiás, Mato Grosso, Mato Grosso do Sul, and Minas Gerais [27] in the Brazilian savannah (the Cerrado) [28]. The fruits and seeds of the plant are used to prepare soap for the treatment of dermatitis, lice infestations, and as an insecticide and larvicide [29]. Larvicidal activity of ethanolic extracts from the *M. pubescens* stem have been reported against different species of the *Aedes* genera, the main vector of the dengue disease [30]. There is also a report on the leishmanicidal activity of the stem bark ethanolic extract [31]. The volatile and flavonoid profile in flowers and leaves from *M. pubescens* have also been investigated, suggesting it could be effective against free radicals [32]. In addition, essential oils from *M. pubescens* inflorescences and their cytotoxic activity have been studied [33]. Infusions of the roots are also used as a tranquilizer, and its ethanolic extracts are used to treat wounds and pain [34]. This extract shows inhibitory activity against insects, fungi, and bacteria. In addition, the ethanolic extract of *M. pubescens* roots displays strong cytotoxic activity against MDA-MB-435 breast cancer cell lines (IC$_{50}$ 7.9 µg/mL) [29]. Furthermore, a study on the insecticide irritability effect of the ethanol extract from the roots of *M. pubescens* demonstrated that this induced significantly greater insecticide-irritability behavior than the control, permethrin, against *Sitophilus zeamais* in stored maize [35]. An analysis of the external coat mucilage from *M. pubescens* seeds has also been reported [36].

Despite the potential of *M. pubescens*, limited studies have been performed on this plant. The goal of the present study is to investigate the potential of *M. pubescens'* roots extract as a biopesticide. Herein, the evaluation of the ethanolic extract of the roots from this species on the phytopathogenic fungi, *Botritis cinerea*, *Fusarium oxysporum*, and *Alternaria alternata* is reported. In addition, the insecticide effect of the plant was evaluated on the *Chrysodeixis chalcites* insect, responsible for plant diseases in economically important crops. The results of this study highlight the potential of *M. pubescens* as a source of eco-friendly biopesticides to suppress highly phytopathogenic species that cause crop losses worldwide.

2. Materials and Methods

2.1. Chemicals and Reagents

All solvents used were analytical grade (Panreac, Barcelona, Spain). Polygram Sil G/UV$_{254}$ foils used for analytical thin-layer chromatography (TLC) were purchased from Macherey-Nagel. A PGA culture medium (potato glucose agar, Sigma Aldrich, St. Louis, MO, USA) and 9 cm diameter petri dishes from Sarstedt (Nümbrecht, Germany) were used for the maintenance of fungal colonies and the performance of bioassays. Tetracycline (50 mg/L, Sigma Aldrich, St. Louis, MO, USA) was added to avoid bacterial growth on petri dishes. Agarose (Sigma Aldrich, St. Louis, MO, USA, 30 g/L) was used for insect bioassays. Stock solutions of plant extracts/fractions were prepared with absolute ethanol (Sigma Aldrich, St. Louis, MO, USA).

2.2. Plant Material

Roots of *M. pubescens* A.St.-Hil. were collected in the municipality of Montes Claros (geographical coordinates: 16°25′26″ S, 43°32′10″ O) in the north of Minas Gerais, Brazil in October 2019. A voucher specimen was deposited at the University Federal Minas Gerais (UFMG) herbarium, under No. 106750. The plant material was registered at Conselho de Gestão do Patrimônio Genético (CGEN/SisGen), under voucher number AAC6C0E.

2.3. Plant Extract Preparation

The air-dried, powdered roots of *Magonia pubescens* (1.0 kg) were extracted by exhaustive maceration (four times) with 98% ethanol (10 mL of ethanol/g of plant material) at room temperature for 94 h for each maceration process. The extractive solvent was removed under reduced pressure at 40 °C using a rotary evaporator, yielding 123 g of residue extract. An aliquot of the extract was used for antifungal (30 mg) and antifeedant (150 mg) evaluation.

2.4. Liquid–Liquid Partition Procedure

The ethanolic plant extract was further fractionated with different solvents. Thus, they were sequentially fractionated by liquid–liquid partition using a series of solvents of increasing polarity by a modification of the Kupchan method [37]. Subsequently, the crude extract was dissolved in a mixture of methanol/water (2:8) and fractionated with hexanes (500 mL), dichloromethane (500 mL), ethyl acetate (4 × 500 mL), *n*-butanol (4 × 500 mL), and water (4 × 500 mL). The organic phases were dried with Na$_2$SO$_4$, filtered, and concentrated under reduced pressure to afford the corresponding organic fractions. The aqueous phase was lyophilized, thereby yielding the aqueous fraction. A preliminary Thin Layer Chromatography (TLC) analysis of the fractions revealed a similar phytochemical profile of the hexane and dichloromethane fractions, and these were combined. Thus, fractions extracted with hexane/dichloromethane (8.7 g), ethyl acetate (46.0 g), *n*-butanol (47.6 g), and water (18.4 g) were obtained, and an aliquot of each was used for biological evaluation.

2.5. In Vitro Test-Assay on Mycelium

Phytopathogenic fungi for bioassays belong to three cosmopolitan genera that cause serious crop damage: *Alternaria alternata*, *Botrytis cinerea*, and *Fusarium oxysporum*. These

fungi were maintained at 25 °C in darkness and periodically replicated in petri dishes with a PGA culture medium and tetracycline to avoid the proliferation of contaminations. Strains of *B. cinerea* (B05.10) and *A. alternata* (Aa 100) were isolated from *Vitis vinifera* and *Lycopersicon esculentum*, respectively, both supplied by the Universidad de La Laguna, Tenerife. The *F. oxysporum* f. sp. lycopersici (2715) strain, isolated from *Lycopersicon esculentum*, was provided by the Colección Española de Cultivos Tipo (CECT) from Valencia, Spain.

Antifungal activity was analyzed through mycelial growth inhibition using an agar-dilution method [38]. Once the culture medium solidified, eight 4 mm diameter discs of the target fungus per dish were placed in test and control petri dishes. One dish per pathogen for each extract with eight sub-replicates in each dish was used. A stock solution of 50 mg/mL was prepared, using ethanol as the solvent, and samples were assayed at a concentration of 1 mg/mL. Colonies grown on petri dishes were incubated for 48 h for *B. cinerea* and 72 h for *A. alternata* and *F. oxysporum* and were digitalized and measured with the application ImageJ. Percent inhibition (% I) was calculated as $\% I = [(C - T)/C] \times 100$, where C is the diameter of the control colonies and T is the diameter of the test colonies. Samples were tested at 1 mg/mL, and those showing a % I higher than 20% at a concentration of 1 mg/mL were then tested at lower doses (0.5 and 0.1 mg/mL). Fosbel Plus (Probelte S.A., Murcia, Spain) was used as a reference fungicide [39]. It is a commercial product purchased in a phytosanitary product store, whereas ethanol was used as a negative control, using one dish per pathogen and eight discs for each control.

2.6. Leaf-Disk Bioassay

A laboratory colony of *Chrysodeixis chalcites* was initiated using larvae collected from banana crops in Tenerife. The larvae were reared on an artificial diet [40] at 21 °C, 70% relative humidity, and a photoperiod of 16:8 h (L:D) in a growth chamber. Adults were fed 30% w/v with diluted honey [41], and the newly fifth-instar larvae of *C. chalcites* was used in the antifeedant bioassays. Indoxacarb 30% (Steward 30WG, FMC Agricultural Solutions, Madrid, Spain) [42], a commercial insecticide, was used as a positive control for comparative purposes.

The bioassay was based on the protocol described by Escoubas et al. [43] and González-Coloma et al. [44], with modifications. Thus, petri dishes with a banana leaf (grown in a greenhouse free of chemical treatments) and a 5 mm deep agarose layer, which delineates intake zones and prevents the leaf disk from drying out during the assay, were prepared. Each petri dish had nine circular holes (1 cm diameter each) so that the larvae could access the leaf. The leaves were previously cleaned with distilled water and left to dry. In the choice assay, leaf disks (5 cm diameter) were alternatively treated with the tested sample solution in ethanol (5 μL per disk–0.2 mg/cm^2), or solvent as the control. In the non-choice assay, all disks of the same plate were treated with the test solution at the same concentration or solvent. After complete evaporation of the solvent, three fifth-instar larvae of *C. chalcites* were placed in each petri dish (six replicates for each experiment) and allowed to feed in a growth chamber. The duration of the bioassay under these conditions was 20 h. The results were expressed in terms of refusal rate (FR) [45]. To calculate the FR in each dish, the leaf area consumed was measured with the ImageJ 1.53e program [46], and the refusal rate (FR) was calculated using the following formula: FR = [1 − (% Treated disks consumed/% Control disks consumed)] × 100.

2.7. Statistical Analysis

All data were shown as a mean ± standard deviation (SD). In the test-assay on mycelium, the percentage of inhibition was analyzed using one-way analysis of variance (ANOVA). Tukey HSD multiple comparisons of means was used to compare concentrations and treatments. Differences of $p < 0.05$ were considered statistically significant. All the analyses were performed using Social Science Statistics, 2018.

Leaf-disk bioassay, using three fifth-instar larvae of *C. chalcites*, were conducted in sextuple for each experiment. The results were expressed in terms of refusal rate (FR),

and the leaf area consumed was measured with the ImageJ 1.53e program. Differences of $p < 0.05$ were considered statistically significant. The analysis of variance was determined by one-way ANOVA [47].

3. Results and Discussion

3.1. Antifungal In Vitro Test-Assay on Mycelium

First, the ethanolic extract of the roots from *Magonia pubescens* was evaluated against *Botrytis cinerea*, *Fusarium oxysporum*, and *Alternaria alternata* (Figures 1–4 and Table S1 in Supplementary Materials), phytopathogenic fungi that affect crops worldwide, in particular, banana and tangerine crops, which are of economic relevance in Brazil. The evaluation of this ethanolic extract (EE) showed a percentage inhibition (% GI) of 30.90, 33.63, and 31.88 against *A. alternata*, *B. cinerea*, and *F. oxysporum*, respectively, at a concentration of 1 mg/mL. These results suggest some degree of antifungal activity against the tested fungi. Subsequently, in order to enrich this extract in bioactive components, the ethanolic crude extract was subjected to a liquid–liquid partition into five fractions, hexanes (F-H), dichloromethane (F-D), ethyl acetate (F-E), *n*-butanol (F-B), and water (F-W) fractions. A preliminary Thin Layer Chromatography (TLC) analysis of these fractions revealed similar phytochemical profiles of hexanes and dichloromethane fractions, and these were combined (F-H/D). These fractions were further evaluated against the three phytopathogenic fungi. The extract and fractions were first tested at a concentration of 1 mg/mL, and those exhibiting growth inhibition higher than 20% were tested at lower concentrations, namely, 0.5 and 0.1 mg/mL. Fosbel Plus was used as a reference fungicide [39], whereas ethanol was used as a negative control, using one dish per pathogen and eight disks for each control.
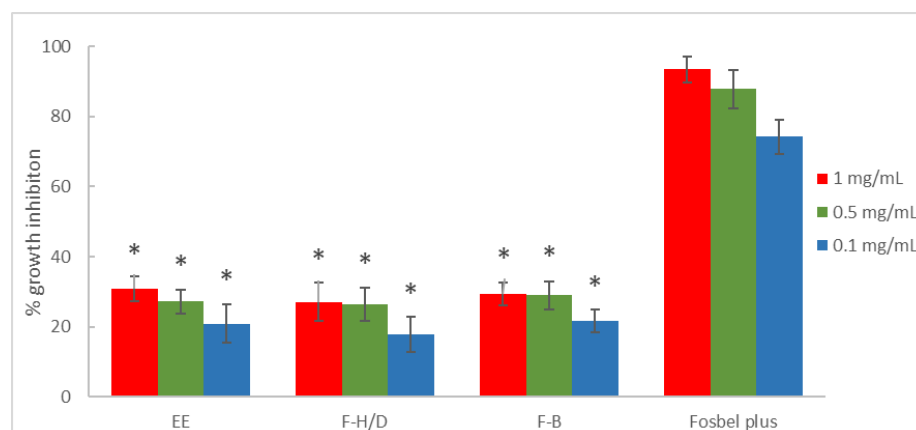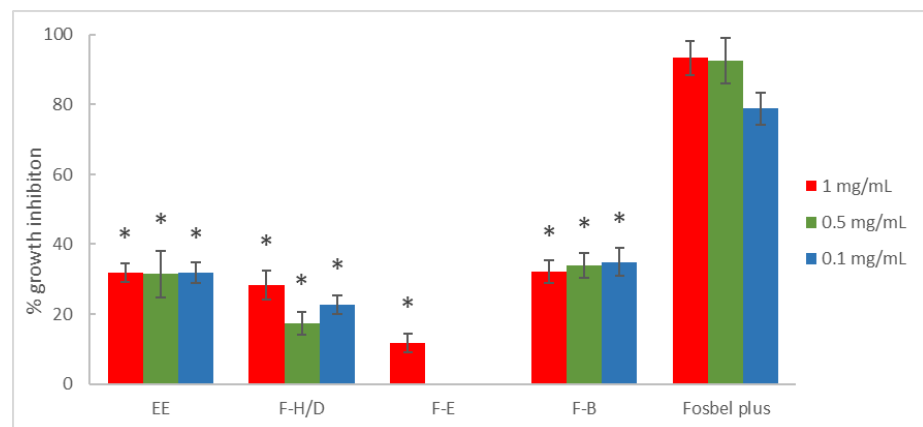

Figure 1. Antifungal effects (% growth inhibition, % GI) of the ethanolic extract (EE) and fractions (F-H/D: hexane/dichloromethane and F-B: *n*-butanol) from *M. pubescens*' roots against *A. alternata*. Fosbel Plus was used as a positive control. Results are expressed as a percentage relative to the negative control. Data are presented as means ± SD (standard deviation, *n* = 8); $p < 0.05$ (*). F-E (ethyl acetate) and F-W (water) fractions were inactive (GI < 10%). Extract/fractions with a GI > than 20% at 1 mg/mL were assayed at lower concentrations (0.5 and 0.1 mg/mL).

Results obtained on *A. alternata* (Figure 1) revealed that fractions F-H/D and F-B (% GI 27.10 and 29.35, respectively) showed similar potency to the crude extract (% GI 30.90), whereas the F-E and F-W fractions were inactive (GI < 10% at 1 mg/mL concentration). Thus, this procedure resulted in maintaining the activity of the two organic fractions, hexanes/dichloromethane and *n*-butanol fractions, in comparison with the ethanolic extract at all the assayed concentrations but did not result in an enrichment in bioactive components.
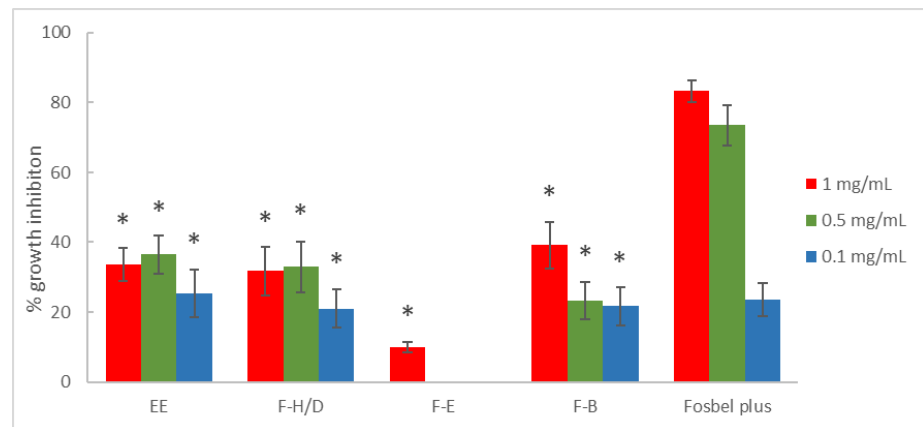
Figure 2. Antifungal effects (% growth inhibition, % GI) of the ethanolic extract (EE) and fractions (F-H/D: hexane/dichloromethane, F-E: ethyl acetate, and F-B: *n*-butanol) from M. pubescens' roots against B. cinerea. Fosbel Plus was used as a positive control. Results are expressed as a percentage relative to the negative control. Data are presented as means $\pm$ SD (standard deviation, $n = 8$); $p < 0.05$ (*). F-W (water) fraction was inactive (GI < 10%). Extract/fractions with a GI > than 20% at 1 mg/mL were assayed at lower concentrations (0.5 and 0.1 mg/mL).

Figure 3. Antifungal effects (% growth inhibition) of the ethanolic extract (EE) and fractions (F-H/D: hexane/dichloromethane, F-E: ethyl acetate fraction, and F-B: *n*-butanol) from *M. pubescens'* roots against *F. oxysporum*. Results are expressed as a percentage relative to the negative control. Fosbel Plus was used as a positive control. Data are presented as means $\pm$ SD (standard deviation, $n = 8$); $p < 0.05$ (*). F-W (water) fraction was inactive (GI < 10%). Extract/fractions with a GI > than 20% at 1 mg/mL were assayed at lower concentrations (0.5 and 0.1 mg/mL).

Likewise, antifungal activity of the ethanolic extract and fractions on B. cinerea (Figure 2) led to a similar effect of the F-H/D fraction (% GI 31.74 at 1 mg/mL) and a slight increase in activity of the F-B fraction (% GI 39.18 at 1 mg/mL) compared to the ethanolic extract (% GI 33.63 at 1 mg/mL). However, the F-E and F-W showed low potency to inactivity with respect to the EE.

F. oxysporum (Figure 3) showed a similar behavior to the extract and fractions as the other two assayed fungi. Thus, the F-H/D (% GI 28.34) and F-B (% GI 32.15) fractions exhibited similar effects as the ethanolic extract (% GI 31.88), whereas the F-E and F-W fractions were almost inactive (GI around or lower than 10% at 1 mg/mL).
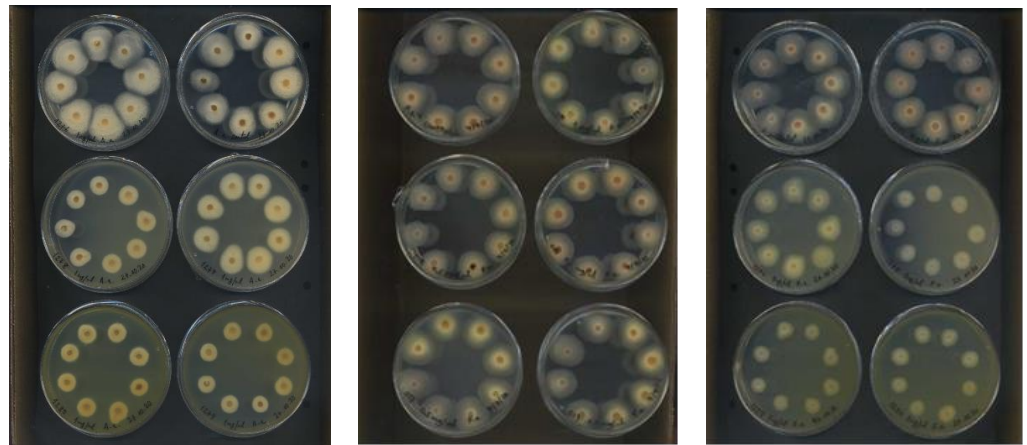
Figure 4. Petri dishes of the in vitro test-assay on *A. alternata*, *B. cinerea*, and *F. oxysporum*.

The F-W fraction showed low to non-activity on the three phytopathogenic fungi, revealing that the components in the plant responsible for antifungal activity have medium to low polarity. Moreover, although none of the fractions exhibited higher growth inhibition than Fosbel Plus, used as a positive control, these potential biopesticides are expected to be less persistent and harmful than synthetic ones, making their use more desirable.

3.2. Insect Antifeedant Activity against Chrysodeixis chalcites

The ethanolic extract and fractions from *M. pubescens'* roots were evaluated on *Chrysodeixis chalcites*, an insect that causes severe damage to crops worldwide. In this assay, samples were dissolved in ethanol, and 5 µL from a stock solution of 40 mg/mL per disk 0.2 mg/cm^2 were used. Indoxacarb 30% (Steward 30WG), a frequently used synthetic insecticide, was used as a positive control, whereas a solvent was used as a negative control. Under the experimental conditions described in the previous section, in the choice assay and the non-choice assays, three five-instar larvae of *C. chalcites* were placed in each petri dish (six replicates for each experiment), and after a 20 h period, the ingested leaf area was measured, and the refusal rate (FR) was calculated. A value of FR > 50% in the choice or non-choice assays indicates a significant feeding inhibition [44]. The combination of FR values > 50% at a dose of 0.2 mg/cm^2 in both assays indicates a strong antifeedant effect. Samples tested with a combination of FR < 50% were considered inactive (Table 1, and Figure 5).

Table 1. Insect antifeeding activity (% FR) [1] of the ethanolic extract and fractions from *Magonia pubescens'* roots against *Chrysodeixis chalcites*.

Sample [2]	Choice Feeding Assay	Non-Choice Feeding Assay	Antifeedant Category
EE [3]	92.14	95.16	strong insect antifeedant
F-H/D [3]	60.52	−27.67	weak insect antifeedant
F-E [3]	−108.09	61.10	appetite suppressor
F-B	−6.69	60.71	appetite suppressor
F-W [3]	−74.57	44.73	inactive
C [4]	97.95	99.04	strong insect antifeedant

[1] Values are expressed as a refusal rate (FR) = [1 − (% Treated disks consumed/% Control disks consumed)] × 100. [2] Samples were dissolved in ethanol: 5 µL per disk–0.2 mg/cm^2. [3] EE: ethanolic extract, F-H/D: hexane-dichloromethane fraction, F-E: ethyl acetate fraction, FB: *n*-butanol fraction, F-W: water fraction. [4] C: indoxacarb 30% (STEWARD® 30WG) was used as a positive control.

Figure 5. Petri dish with *Chyxodeixis chalcites* larvae in the leaf-disk bioassay.

The results indicate that the crude extract (EE) had a FR > 90% in the choice and non-choice assays, meaning that the extract acts very effectively as an antifeedant for *C. chalcites* larvae. Subsequently, hexane/dichloromethane (F-H/D), ethyl acetate (F-E), *n*-butanol (F-B), and water (F-W) fractions obtained by liquid–liquid partition of the ethanolic crude extract were further evaluated against *C. chalcites*. The results revealed that the hexane/dichloromethane fraction (F-H/D) acts as a weak insect antifeedant, since in the choice assay, larvae preferred to feed on untreated disks (FR = 60.52%), indicating good activity, but in the non-choice assay, the larvae fed from the treated disks (FR = −27.67%), so it does not have a complete antifeedant effect. Moreover, the ethyl acetate (F-E) and *n*-butanol (F-B) fractions showed positive FR values in the non-choice assay (FR 61.10% and 60.71%, respectively), though activity was not confirmed by the choice assay (low FR values). Thus, the F-E and F-B fractions are appetite suppressants since larvae do not feed off treated disks in the non-choice assay. On the other hand, the water (F-W) fraction was inactive, showing FR values of −74.57 and 44.73%, respectively, in the assays.

Notably, the crude ethanolic extract showed similar effects as Steward 30WG [42], the commercial insecticide used as a positive control, exhibiting an FR of 92.14% and 95.16% in the choice and non-choice feeding assays, respectively. This fact suggests that the plant could be used, after a simple and accessible procedure, as a biopesticide against this phytopathogenic insect. The only study regarding the potential insecticide activity of *M. pubescens* is by Silva et al. [35], who reported the insecticide irritability effect (avoidance after contact) similar to permethrin in its stem ethanolic extract against *Sitophilus zeamais* (maize weevil), a serious pest of stored maize. However, the present study is the first report regarding the antifeedant potential of *M. pubescens*' roots. Furthermore, several chemical components have been identified in different parts of the plant, including tannins, isolated from the steam bark [48] and 2-*O*-methylinositol and proanthocyanidin from the peel of the fruits [49]. Moreover, flavonoids and volatile oil constituents of its flowers and leaves [32] and essential oils from inflorescences [33] have been reported. In addition, a preliminary phytochemical survey allowed the identification of steroids, tannins, alkaloids, saponins, and flavonoids in the leaves and flavonoids and alkaloids in the bark of the tree trunks [50]. However, a phytochemical analysis of the roots has not been reported. The findings reported herein highlight the effect of *M. pubescens*' roots on phytopathogen insects and deserve future phytochemical investigation to identify the metabolites responsible for this effect with a view to developing biopesticides.

4. Conclusions

In summary, the ethanolic extract from *Magonia pubescens*' roots was moderately efficient at suppressing the mycelial growth of the phytopathogenic fungi, *Alternaria alternata*, *Brotrytis cinerea*, and *Fusarium oxysporum*, with a percentage of inhibition around 30 at

1 mg/mL. The liquid–liquid partition led to organic fractions exhibiting a similar potency to the ethanolic extract, whereas the aqueous fraction showed low to non-activity on the three phytopathogenic fungi, indicating that the antifungal components possess ranges from low to medium polarity.

Furthermore, the ethanolic extract displayed a strong insect antifeedant activity against *Chrysodeixis chalcites* larvae, showing refusal rate values higher than 92% in both the choice and non-choice feeding assays, comparable to the commercial insecticide used as a positive control, Steward 30WG. However, the organic fractions from the liquid–liquid fractionation behave as appetite suppressors since larvae do not feed off treated disks in the non-choice assay, suggesting a synergism of constituents in the ethanolic extract.

Overall, *M. pubescens*' roots could be applied in combination with other biological agents in an environmentally safe and economically acceptable integrated management of crop diseases, especially against the *C. chalcites* insect. However, additional research is required to optimize extraction procedures and corroborate these results in field trials.

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