



Antifungal Effect of Different Plant Extracts against Phytopathogenic Fungi *Alternaria alternata* and *Fusarium oxysporum* Isolated from Tomato Plant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The antifungal activity of 10 plant extracts was tested against the phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*, the pathogens of early blight and wilt diseases of tomato. Plants tested related to different families. Three doses (10, 50 and 100 mg/ml) of plant extracts were assayed on *A. alternata* and *F. oxysporum* by measuring the inhibition zone of mycelial growth of fungi by disc-diffusion method on the Potato dextrose agar (PDA). The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of plant extracts were determined. The inhibitory effect of plant extracts ranged from 10-29 and 12-31 mm against *A. alternata* and *F. oxysporum*, respectively. The extracts of *Pulicaria crispera* and *Olea chrysophylla* were the most effective antifungal followed by *Citrullus colocynthis*, *Psiadia arabica* and *Otostegia fruticosa*. These plant extracts contain biologically active major compounds such as alkaloids, flavonoids, saponins, tannins and terpenoids. The remaining plant extracts did not emerge any inhibitive effect on tested fungi. The MIC and MFC of plant extracts ranged 6-38, 7-34 and 28-56,

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22-50 mg/ml for *A. alternata* and *F. oxysporum*, respectively. Therefore, these plant extracts have biologically active compounds and have a potential to control fungal phytopathogens in agricultural crops.

Keywords: Antifungal activity; plant extracts; *Alternaria alternata*; *Fusarium oxysporum*; phytopathogenic fungi; fungicides, fungitoxic properties; plant pathogenic fungi

1. INTRODUCTION

Alternaria and *Fusarium* fungi are well known plant pathogens that cause significant productivity losses in agricultural crops all over the world. *A. alternata* and *F. oxysporum* are causing early blight and wilt of tomato [1,2]. Usually, synthetic fungicides are used to control of pathogens. It is considered the most pollutant for the environment in addition its toxicity to consumers. Plant extracts are safe, healthy and environmentally friendly alternatives to synthetic fungicides. These natural products can be used in plant production because of specific toxicity to filamentous fungi without destroying non-target organisms [3,4]. Some plant families have fungicidal activity against *Alternaria* and *Fusarium* species such as Asteraceae [3,5], Oleaceae [3,6], and Lamiaceae [3,7]. Mohana et al. [8] assayed the antifungal activity of 12 methanol plant extracts belong to different families against eight seed-borne pathogenic fungi, *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Drechslera oryzae*, *D. halodes*, *Fusarium moniliforme*, *Pyricularia oryzae* and *Trichoconis padwickii*. The methanol extracts of *Acacia nilotica*, *Caesalpinia coriaria*, *Decalepis hamiltonii*, *Embllica officinalis*, *Lawsonia inermis* and *Mimosops elengi* showed significant antifungal activity at 3500 µg/ml. In order to decrease the use of synthetic fungicides, extensive investigations were performed for the possible use of plant natural products as commercial antifungal or fungicides for the control of fungal pathogens [8]. The antifungal activity of plant extracts applied on the *Fusarium oxysporum* f. sp. *lycopersici* development. Among 500 plant species investigated, 84% did not emerged significant inhibitive effect, however 7.6% appeared low inhibitive effect and 5.2% was moderate for antifungal activity. 3% only was completely inhibited the germination of fungi. These findings emerged that plant extracts which possess antifungal activities toward *Fusarium oxysporum* could be employed as antifungal agents for the control of plant fungal diseases [3]. Recently, Lira-De León et al. [5] studied the antifungal activity of 12 plant extracts toward the *Alternaria alternata* and *Fusarium solani*

phytopathogens. Plants were collected from Oaxaca State and related to families: Asteraceae, Boraginaceae, Convolvulaceae, Crassulaceae, Euphorbiaceae, Nyctaginaceae, Orchidaceae, Rubiaceae, Tiliaceae and Verbenaceae. The antifungal effect of different doses of plant extracts (50–100 mg/ml) toward *A. alternata* and *F. solani* was estimated by detection of mycelium radial growth and obtained the minimum inhibitory concentration (MIC) of tested fungi. The inhibitive effect of plant extracts on mycelial growth of fungi ranged 2.02-69.07 and 0.76-56.17% for *A. alternata* and *F. solani*, respectively. The extracts of *A. subviscida*, *I. murucoides*, *L. achyranthifolia* and *T. densiflora* emerged MIC values ranged 5.77-12.5 mg/ml. *A. aurantium* emerged the best inhibition for *A. alternata* (68.64% MIC=7.78 mg/ml) and *F. solani* (56.17%, MIC=7.78 mg/ml) [5]. The antifungal effect of crude plant extracts against *Alternaria alternata* and *Fusarium oxysporum* were also detected in several parts of the world [9,10,11,12,13]. The phytochemical analysis of ethanolic extracts of plant leaves emerged the presence of antifungal compounds with variable levels, such as alkaloids, flavonoids, saponins, triterpenoids, and tannins [14]. They have medicinal importance due to their activities as antimicrobial and medicinal effects [15,16,17]. The present study planned to evaluate the antifungal activity of plant extracts against phytopathogenic fungi, *A. alternata* and *F. oxysporum* to find an alternative safe biological control. In addition, it planned to phytochemical screening of plant extracts for their biologically active compounds.

2. MATERIALS AND METHODS

2.1 Isolation of Fungi

Alternaria alternata was isolated from early blight disease symptoms of tomato leaves. The infected leaves were rinsed with sterilized distilled water followed by immersion in 1% sodium hypochlorite for 1 minute. Then the leaves were rinsed several times with sterilized distilled water and dried using absorbent paper. Fragments of 2 cm² each were cut and placed on

the Potato dextrose agar (PDA) plates and incubated at 28°C for 7 days. However, *Fusarium oxysporum* was isolated from rhizoplane of tomato plant. The roots were rinsed several times with sterilized distilled water and dried by absorbent paper. The roots were cut into 2 cm segments and placed on the PDA plates. Plates were incubated at 28°C for 7 days. The developed *Alternaria alternata* and *Fusarium oxysporum* were isolated, identified based on morphological characteristics of the colonies, mycelium and conidia using the key of imperfect fungi and employed in this investigation [18].

2.2 Medium Used for Isolation of Fungi

Potato dextrose agar medium which contained potatoes 200, dextrose, 20 and agar, 15 g was used for isolation of *Alternaria alternata* and *Fusarium oxysporum* fungi (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Amoxicillin (0.5 mg/ml) was used as bacteriostatic agent.

2.3 Collection of Medicinal Plants

Ten wild plants (*Bupleurum falcatum*, *Citrullus colocynthis*, *Dodonaea viscosa*, *Ficus palmate*, *Olea chrysophylla*, *Otostegia fruticosa*, *Psiadia arabica*, *Pulicaria crispa*, *Rumex vesicarius* and *Zygophyllum simplex*) related to 9 different families (Apiaceae, Cucurbitaceae, Sapindaceae, Moraceae, Oleaceae, Lamiaceae, Asteraceae, Polygonaceae and Zygophyllaceae) were collected from Alaqeeq Valley, 25 km south Almadinah Almonawarah, Saudi Arabia on November 2018. Plants were identified by a specialist in plants taxonomy Dr. Usama Kamal, Biology Department, Taibah University, Saudi Arabia. A voucher specimen from each plant used in the study was deposited at the plant laboratory of the above department. Plant scientific name, phytochemicals and medicinal uses are listed in Table (1).

2.4 Preparation of Crude Extracts of Plants

Plant materials were air dried in the shade and then the leaves were grounded using a Wiley grinder to fine powder. 200 g of the powder was refluxed in 1 Liter of 70% ethanol at 50°C for 48 hours in continuous extraction (Soxhlet apparatus). Ethanol extract was filtered and concentrated under reduce pressure at 40°C using a rotary evaporator. The residue (active principles) was stored at -4 until further use.

2.5 Determination of Chemical Constituents of the Ethanolic Plant Extracts

Gas Chromatography-Mass Spectroscopy (GC-MS) (AGLENT GC-MS and NIST Library) was used to determine the chemical composition of plant extracts. The biologically active substances were investigated and identified in various plant extracts depend on GC retention time on Rtx®-5MS fused silica capillary column. The mass spectra were matched with computer program in related to those of standards (NIST 2005 v.2.0 and Wiley Access Pak v.7, 2003 of GC-MS systems), by co-injection matched with authentic compounds [18].

2.6 Antifungal Activity of Plant Extracts

The antifungal activity of the ethanolic extract of plants was determined by disc diffusion assay as described by Aouadhi et al. [19]. *Alternaria alternata* and *Fusarium oxysporum* were cultivated in Petri plates containing PDA medium. The paper discs (6 mm diameter) were separately applied with 20 µl of the three doses (10, 50, and 100 mg/ml) of evaluated plant extracts and placed on the agar which had previously inoculated with tested fungi. Disc with sterilized distilled water was used as control. Plates were incubated for 5 days at 28°C. Antifungal activity was determined by measuring the diameter of the inhibition zone around disc in millimeter. The measurements of the inhibition zones were carried out three times and the averages were calculated with the three replicates.

2.7 Determination of MIC and MFC of Plant Extracts

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were also determined using a broth dilution method [20]. All antifungal tests were performed in Potato dextrose broth (PDB). Serial dilutions of plant extracts, ranging from 1 to 60 mg/ml, were used. Tubes were inoculated with 1 ml of spore suspension of tested fungi and incubated for 72 hrs at 28°C. Then the MICs and MFCs were determined according to changes in optical density following conidial germination after 72 hrs followed the inoculation using UV-Spectrophotometer (Model AE-450, 2003, Japan). Fungal growth was indicated by the presence of turbidity and a 'pellet' on the tube bottom. The MIC value was determined as the lowest concentration of plant extract that

inhibited the visible growth of the pathogenic fungi, while the MFC was the concentration where 99% or more of the initial inoculum was killed. Each assay was prepared in triplicate.

2.8 Statistical Analysis

Statistical analysis of data was carried out by one-way analysis of variance. The means and standard deviations were separated by Tukey's honest significant difference test using Biostat 2008 statistical analysis program (Copyright ©2001-2009 Analystsoft).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of Plant Extracts

Phytochemical analysis of 10 plant extracts by Gas Chromatography-Mass Spectroscopy (GC-MS) emerged the presence of biologically active major compounds such as alkaloids, flavonoids, saponins, tannins and terpenoids (Tables 1 and 2). These compounds exhibited an inhibitive effect against plant pathogens *A. alternata* and *F. oxysporum*. Studies on natural products have been accelerated in recent years due to their importance in drug discovery and antimicrobial activity. Several plant molecules with antifungal activity toward different pathogenic fungi have been found, which are highly important to human. Some of these plant molecules could be employed directly or used as intermediates for developing antifungal compounds [19]. Plants are rich source of wide types of bioactive secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, saponins and other compounds. *In vitro*, these compounds have antifungal properties [14]. Recently, Ali [17] screened the phytochemicals of *Epipremnum aureum* by GC-MS. Twenty-one organic compounds were detected with different retention times. They were carbohydrates, fatty acids, phenols, alcohols, vitamins, alkaloids and flavonoids. Patchoulol represented the highest percentage of phytochemicals followed by myristic and palmitic acids [17].

3.2 Antifungal Activity of Plant Extracts

The antifungal activity of 10 plant extracts was tested against the phytopathogenic fungi, *Alternaria alternata* and *Fusarium oxysporum*, the popular pathogens of both early blight and wilt diseases of tomatoes. Plants tested related to different families (Apiaceae, Cucurbitaceae,

Sapindaceae, Moraceae, Oleaceae, Lamiaceae, Asteraceae, Polygonaceae and Zygophyllaceae). The antifungal activity of three doses of plant extracts (10, 50 and 100 mg/ml) against *A. alternata* and *F. oxysporum* was estimated by measuring the inhibition zone of mycelial growth of fungi by disc diffusion method on Potato dextrose agar (PDA). The results obtained showed that the inhibitory effect of plant extracts ranged 10-29 and 12-31 mm against *A. alternata* and *F. oxysporum*, respectively. The extracts of *Pulicaria crispera* and *Olea chrysophylla* were the most effective antifungal followed with *Citrullus colocynthis*, *Psiadia arabica* and *Otostegia fruticosa* extracts. However, the remaining plant extracts did not emerge any inhibitive effect on tested fungi (Table 3). The Minimum inhibitory concentrations (MIC) of plant extracts ranged 6-38 and 7-34 mg/ml for *A. alternata* and *F. oxysporum*, respectively. However, the minimum fungicidal concentrations (MFC) ranged 28-56 and 22-50 mg/ml for *A. alternata* and *F. oxysporum*, respectively (Table 4). Al-Rahmah et al. [21] evaluated the antifungal activity of five methanolic plant extracts on tomato phytopathogenic fungi, *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*. Three of five plant extracts were effective against phytopathogenic fungi. *Thymus vulgaris* and *Zingiber officinale*. The extracts were strongly active and showed fungicidal activity against fungi with MIC of 4 mg/ml and MFC of 8 mg/ml. *F. oxysporum* was less sensitive and its MFC reached 16 mg/ml for *Z. officinale* extract. However, *S. persica* extract showed a moderate antifungal activity, while *L. camara* and *Z. spinachristi* were not effective against tomato phytopathogenic fungi [22]. Shaban et al. [13] screened the antifungal activity of *Cuminum cyminum* and *Foeniculum vulgare* extracts against *Rhizoctonia solani*, *Fusarium oxysporum* and *Fusarium moniliforme*. All plant extracts significantly inhibited the growth of these pathogenic fungi. They also showed that 75% concentration of plant extract was the most effective. The plant extracts had different levels of antifungal activity against the tested pathogenic fungi. Dellavalle et al. [23] evaluated the antifungal activity of 10 Uruguayan medicinal plant extracts toward the *Alternaria* spp. Phytopathogen. The plant acid extracts were more effective than the buffer or aqueous extracts toward *Alternaria* spp. The MIC of the extracts ranged 1.25-25 µg/ml. However, MFC of the extracts ranged 1.25-10 µg/ml. Abu-Taleb et al. [24] screened *Rumex vesicarius* and *Ziziphus*

Table 1. Scientific names, phytochemicals and medicinal uses of plants

Scientific name	Family	Phytochemicals	Medicinal uses
<i>Bupleurum falcatum</i>	Apiaceae	Tannins, phenols, proteins, cardiac glycosides, saponins (saikosaponins) and steriods.	Anti-inflammatory and antioxidant agent, antiulcerative, hepatoprotective and nephroprotective activities.
<i>Citrullus colocynthis</i>	Cucurbitaceae	Alkaloids, flavonoids, cardiac glycosides, proteins, saponins, steriods and terpenoids.	Antioxidant, antimicrobial and antimalarial, hepatoprotective, antispermatogenic and carcinogenic. It is used to treat diabetes, improving lipid levels and reduce cytokine.
<i>Dodonaea viscoa</i>	Sapindaceae	Flavonoids, saponins, proteins, steriods and tannins.	Treatment fever and malaria, relieve itching, fevers swellings, aches, antispasmodic agent, as toothaches and headaches, digestive system disorders including indigestion, ulcers and diarrhea.
<i>Ficus palmata</i>	Moraceae	Alkaloids, proteins, terpenes, cardiac glycosides and tannins.	Antitumor, anti-inflammatory and tonic medicament microbial diseases such as epilepsy and jaundice, bronchitis, influenza whooping cough, tonsillitis, toothache, bacillary dysentery, enteritis and bruises.
<i>Olea chrysohylla</i>	Oleaceae	Alkaloids, flavonoids, terpenoids (diterpenes, triterpenes), proteins, cardiac glycosides, saponins, tannins and steriods.	Antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic, and hypocholesterolemic properties. Anticarcinogenic and antiatherosclerotic.
<i>Otostegia fruticosa</i>	Lamiaceae	Alkaloids, flavonoids (morin, kaempferol and quercetin), phenols, tannins, saponins and terpenoids (diterpenes, triterpenes).	Treating arthritis, gastric discomfort, headache, rheumatism, sedative activities, regulating blood pressure and hyperlipidemia. Antimicrobial, antioxidant activities, anti-inflammatory, antidiabetic and hepatoprotective agent.
<i>Psiadia arabica</i>	Asteraceae	Alkaloids, flavonoids, saponins, steriods, tannins and terpenoids.	Treatment of abdominal pains, colds, fevers, bronchitis and asthma, for preparation of decoctions or as plaster for immobilising fractures. Antimicrobial, antiplasmodial and anti-inflammatory activities.
<i>Pulicaria crispa</i>	Asteraceae	Alkaloids, proteins, saponins, phenols, tannins, flavonoids, cardiac	In conventional medicine for the cure of heart diseases and as gastroprotective. Treatment inflammation,

		glycosides, terpenoids (diterpenes, sesquiterpenes).	insect repellent and as an herbal tea, galactagogues and antiepileptics.
<i>Rumex vesicarius</i>	Polygonaceae	Phenols, flavonoids, proteins, saponins, tannins and terpenoids.	Treatment of inflammation, bleeding, cough, headache and cancer. Antioxidant against lipid peroxidation.
<i>Zygophyllum simplex</i>	Zygophyllaceae	Alkaloids, flavonoids, phenols, saponins and tannins.	In Arabic region to treat gout, asthma, horny patches of skin. Its seeds are anthelmintic analgesic and anti-inflammatory.

Table 2. Phytochemical analysis of plant extracts

4Plants	Phytochemicals								
	Alkaloids	Cardiac glycosides	Flavonoids	Phenols	Proteins	Saponins	Steroids	Tannins	Terpenoids
<i>Bupleurum falcatum</i>	-	+	-	++	++	+++	+	++	-
<i>Citrullus colocynthis</i>	++	+	++	-	++	++	+	-	++
<i>Dodonaea viscosa</i>	-	-	++	-	+	++	++	++	-
<i>Ficus palmata</i>	++	++	-	-	+	-	-	+	+
<i>Olea chrysophylla</i>	++	++	+++	-	+	++	+	++	++
<i>Otostegia fruticosa</i>	+	-	++	+	-	+	-	+	++
<i>Psiadia arabica</i>	+++	-	++	-	-	+	+	+	+++
<i>Pulicaria crispa</i>	+++	++	++	++	+	+++	-	+++	++
<i>Rumex vesicarius</i>	-	-	+	+	++	+	-	+	+
<i>Zygophyllum simplex</i>	+	-	+	+	-	+	-	+	-

+++ = high concentration; ++ = Moderate concentration; + = Low concentration; - = Absent

Table 3. Antifungal activity (diameter of inhibition zone, mm) of medicinal plant extracts against *Alternaria alternata* and *Fusarium oxysporum*

Doses (mg/ml)	<i>Alternaria alternata</i>			<i>Fusarium oxysporum</i>		
	10	50	100	10	50	100
Plants	M±SD	M±SD	M±SD	M±SD	M±SD	M±SD
<i>Bupleurum falcatum</i>	--	--	--	--	--	--
<i>Citrullus colocynthis</i>	--	15*±0.8	17*±1.1	--	18*±1.4	21*±1.3
<i>Dodonaea viscoa</i>	--	--	--	--	--	--
<i>Ficus palmata</i>	--	--	--	--	--	--
<i>Olea chrysophylla</i>	14*±0.8	21*±1.2	27*±2.1	12*±1.2	17*±1.6	23*±2.6
<i>Otostegia fruticosa</i>	--	10±0.6	14*±0.8	--	14*±0.8	18*±1.2
<i>Psiadia arabica</i>	--	13*±0.9	20*±1.4	--	16*±1.2	23*±1.7
<i>Pulicaria crispa</i>	16*±1.2	23*±1.4	29*±2.6	21*±1.8	26*±2.4	31*±2.6
<i>Rumex vesicarius</i>	--	--	--	--	--	--
<i>Zygophyllum simplex</i>	--	--	--	--	--	--
Distilled water (control)	--	--	--	--	--	--

M = mean; SD = standard deviation; -- = No antifungal activity
Asterisked values are significantly different compared with the control ($P \leq 0.05$)

Table 4. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) (mg/ml) of ethanolic plant extracts against *Alternaria alternata* and *Fusarium oxysporum*

Plants	<i>A. alternata</i>		<i>F. oxysporum</i>	
	MIC	MFC	MIC	MFC
<i>Bupleurum falcatum</i>	--	--	--	--
<i>Citrullus colocynthis</i>	32	56	26	38
<i>Dodonaea viscoa</i>	--	--	--	--
<i>Ficus palmata</i>	--	--	--	--
<i>Olea chrysophylla</i>	8	36	9	28
<i>Otostegia fruticosa</i>	38	52	34	50
<i>Psiadia arabica</i>	26	42	18	34
<i>Pulicaria crispa</i>	6	28	7	22
<i>Rumex vesicarius</i>	--	--	--	--
<i>Zygophyllum simplex</i>	--	--	--	--
Distilled water (control)	--	--	--	--

spina-christi extracts against two root rot pathogens, *Drechslera biseptata* and *Fusarium solani*. Ethanolic extract was the most effective, followed by aqueous layer fraction. Spore production and germination of *F. solani* were influenced by plant extracts more than *D. biseptata*. However, *F. solani* failed to produce conidiospores after treatment with the ethanolic extract of *Z. spina-christi* at 20% dose [23]. The aqueous extracts antifungal effect of *Cannabis sativa*, *Parthenium hysterophorus*, *Urtica dioeca*, *Polystichum squarrosus* and *Adiantum venustum* were also assayed toward *Alternaria solani*, *Alternaria zinniae*, *Curvularia lunata*, *Rhizoctonia solani* and *Fusarium oxysporum* with various concentrations (5, 10, 15 and 20%). Maximum antifungal activity was recorded at 20% of *C. sativa* extract and emerged high inhibitory effect toward *C. lunata* (100%), *A.*

zinniae (59.68%), followed by *P. hysterophorus* leaf extract against *A. solani* (50%) [24]. Recently, Onaran and Sađlam [4] determined the effect of methanol extracts of various plant parts (roots, leaves, flowers, shoots and fruits) of *Phytolacca Americana*, *Prunus laurocerasus*, *Smilax excelsa*, *Rhododendron ponticum* and *Trachystemon orientalis* that were collected from Turkey Black Sea region toward three of economically important plant pathogens of tomatoes and potatoes (*Alternaria solani*, *Botrytis cinerea* and *Rhizoctonia solani*). Different doses of plant extracts (50, 100, 200 and 400 mg/ml) were assayed toward the plant pathogens. The mycelial growth inhibition (MGI) was recorded [4]. All plants tested were significantly appeared antifungal activities. The maximum MGI (84%) was determined for *P. americana* leaf extract against *B. cinerea*. The highest antifungal

activities were observed at 400 mg/ml of *P. laurocerasus*, *T. orientalis*, *P. americana*, *R. ponticum* and *S. excelsa* respectively. The antifungal effect of various plant extracts was also carried out in several locations of the world including *Psiadia* species [25], *Rumex vesicarius* and *Ziziphus spina-christi* [23], *Citrullus colocynthis* [26], *Olea europaea* [27], *Pulcra crispa* and *Scoparia dulcis* [28,29,30,31].

5. CONCLUSION

In attempts to decrease the widespread of unsafe synthetic fungicides, several investigations were performed for the possible employment of plant substances as natural products against fungal pathogens. The present article surveyed the antifungal activity of 10 plant extracts toward tomato phytopathogens, *Alternaria alternata* and *Fusarium oxysporum*. Among different plants, the extracts of *Pulcra crispa* and *Olea chrysophylla* were the most effective antifungal followed with *Citrullus colocynthis*, *Psiadia arabica* and *Otostegia fruticosa*. These plant extracts contained relatively the highest concentrations of antifungal compounds such as saponins, terpenes, flavonoids, alkaloids, phenols and steroids. These plant extracts are safe and good alternative fungicides against phytopathogenic fungi. Therefore, these plant extracts have a potential for the control of plant diseases in agricultural crops.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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