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Antifungal activity of aqueous and organic extracts from *Withania somnifera* L. against *Fusarium oxysporum* f. sp. *radicis-lycopersici*

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Abstract

The aim of this study was to evaluate the *in vitro* antifungal activity of aqueous and organic extracts from native *Withania somnifera* L. leaves, stems, and fruits against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL), the causal agent of Fusarium Crown and Root Rot disease in tomato. Aqueous and organic extracts (used at 1, 2, 3 and 4%) were added to molten Potato Dextrose Agar (PDA) medium. After pathogen challenge, cultures were incubated at 25°C for 5 days. All extracts tested, whatever the concentrations used, showed a strong antifungal activity toward targeted pathogen. FORL response to the different extracts assessed using the poisoned food technique, varied depending on plant organs, concentrations tested and organic solvent used for extraction. For aqueous extracts, fruit extract used at 2% exhibited the highest antifungal potential where FORL growth was decreased by 56.27%, relative to the untreated control, compared to 52 and 45.34% achieved using stem and leaf extracts at 3%, respectively. The highest antifungal activity of organic extracts was registred at the highest concentration used (4%). FORL was found to be more sensitive to fruit extracts than those from leaves and stems. Among the three organic extracts tested, butanolic fractions were the most active against FORL growth. The highest antifungal potential expressed by 62.03% decrease in pathogen radial growth was displayed by butanolic stem extracts applied at 4%. These results indicate that native *W. somnifera* plants may be exploited as potential source of allelochemicals biologically active against FORL.

Keywords: Antifungal activity; Extract; *Fusarium oxysporum f.* sp *radicis- lycopersici;* Mycelial growth; *Withania somnifera*

Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop worldwide and ranked second after potato [1]. In Tunisia, serious economically important losses were attributed to Fusarium Crown and Root Rot (FCRR) disease incited by the soilborne fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) Jarvis & Shoemaker. Main disease signs are plant wilting at fruit ripening stage, cortical rot at the soil level, vascular discoloration of the lower stem and production of conspicuous pinknish masses of conidia along the stem [2]. Losses from FCRR in greenhouse tomato crops have been estimated at up to 90% [3]. Several control measures have been attempted for FCRR management, both for greenhouse and open field crops, which are restricted to soil physical and chemical desinfection and use of pathogen-free transplants. However, complete suppression of FORL from soil is difficult due to the long survival of its resting structures i.e. chlamydospores. Moreover, the most commonly used chemical fungicides, such benomyl or captafol, are not easily biodegradable and persist for years in soil which lead to environment pollution and the development of resistant strains [4]. Thus, research efforts were more focused on biological control. In fact, under Tunisian conditions, some biofungicides based on *Trichoderma harzianum*, *Pythium oligandrum*, *Bacillus subtilis*, *B. pumilus* have decreased FORL mycelial growth by 50 to 73%. *T. harzianun* strain T22 has also reduced disease incidence to 5.5% under greenhouse conditions [5]. In addition, Hibar et al. [3] and Kerkeni et al. [6,7] also showed the FCRR-suppressive effects of some compost extracts and their associated microorganisms.

In modern agriculture, attention was increasingly focused on the development of effective alternatives of natural origin for plant bioagressors' management. Interestingly, wild and cultivated plants are a potential source of biotic and abiotic antifungal agents [8] which are ecofriendly and may act as safe bio-based fongicides. Several previous studies have been focused on use of aqueous and organic extracts from various plant speciess for the control of some phytopathogenic fungi [9,10]. In this regard, few investigations were performed to screen the antifungal properties of some botanical extracts against FORL [11,12].

Antifungal potential of plant-derived allelochemicals has been demonstrated in various previous studies [13-15]. In fact, aqueous and organic extracts from different *Trigonella foenum-graecum* organs were screened for their ability to suppress growth of five phytopathogenic fungi (namely *Botrytis cinerea*, *F. graminearum*, *Pythium aphanidermatum*, *Alternaria* sp., and *Rhizoctonia solani*) where the most active antifungal compounds were detected in methanol fraction from fenugreek leaves and stems [16]. Acetone extracts from *Cestrum laevigatum*, *Nicotiana glauca*, *Solanum mauritianum*, *Lantana camara*, *Datura stramonium*, *Ricinus communis*, and *Campuloclinium macroephalum* were also screened for their ability to suppress ten phytopathogenic

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fungi including *F. oxysporum* [17]. Al-Rahmah et al. [18] reported that methanolic extracts from *Thymus vulgaris* and *Zingiber officinales* were strongly active against *F. oxysporum*, *P. aphanidermatum* and *R. solani* infecting tomato. On the other hand, some *Solanaceae* species showed a conserved position of genes conferring resistance to some plant pathogens. Thus, Cillo et al. [19] reported that wild tomato species (*Solanum* section *Lycopersicon*), have been often employed by breeders as sources for intergressing disease resistance for tomato genetic breeding. Similar antimicrobial activity of wild *Solanaceae* species has been reported in various studies [20-24].

Withania somnifera (Ashwagandha) is a wild *Solanaceae* plant. It is one of the extremely interesting medicinal plants with pharmaceutical applications. Ashwagandha is a valuable drug associated with various therapeutic properties. Recent studies have assessed the anti-stress, anti-inflammatory, anti-tumour, antibiotic, and anticonvulsant activities in this plant. These activities are more attributed to withaferin A and withanone, the two major steroids found in its leaves and roots [8]. Several studies were focused on the efficient antifungal activity of Ashwagandha extracts against clinical fungi. For instance, Sailaja [25] demonstrated the potent antifungal activity of methanolic stem extracts from *W. somnifera* against *Alternaria alternata*, *Curvularia lunata*, and *Candida albicans*. In another study, *W. somnifera* was found to be very effective against human *Apergillus* infections [26]. Mahesh and Satish [27] demonstrated that methanol root, leaf and bark extracts from *W. somnifera* showed a significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, and *Xanthomonas axonopodis* pv. *malvacearum* and *C. albicans* [28]. However, few reports were focused on its antifungal activity against plant pathogenic fungi. In fact, Girich et al. [8] have demonstrated that *W. somnifera* root aqueous extract contains a glycoprotein (WSG) with fungistatic effect toward three phytopathogenic fungi namely *Aspergillus flavus*, *F. oxysporum*, and *F. verticilloides* by inhibiting their spore germination and hyphal growth. Baka [29] showed that aqueous extracts from *W. somnifera* leaves were able to inhibit the mycelial growth and spore germination of some phytopathogenic fungi such *Alternaria brassicae*, *A. solani*, *Botrytis fabae*, *F. oxysporum*, and *Phytophthora infestans*.

In this paper, we investigate the *in vitro* antifungal activity of leaf, stem and fruit aqueous and organic extracts from wild *W. somnifera* plants collected from Tunisia against FORL.

Materials and Methods

Plant material

Fresh and healthy *W. somnifera* leaves, stems, and fruits (Figure 1) were collected from Tunisian littoral, Chott-Mariem (latitude 35°56'20.451"N, longitude E10°33'32.028") in November 2013. Fresh materials were washed thoroughly under running tap water to remove any dust, and were dried at room temperature at 30°C for 72 h and grounded into fine powder before being used for extraction.

Fungal agent

FORL isolate used in this study was originally recovered from tomato plants showing typical symptoms of Fusarium Crown and Root Rot (FCRR) disease. Fungal cultures were grown on Potato Dextrose Agar (PDA) medium and incubated at 25°C for 5 days before being used for antifungal bioassays.

Preparation of aqueous and organic extracts

Aqueous extracts were prepared by soaking 30 g of dried leaf, stem and fruit powder from *W. somnifera* in 100 ml of sterilized distilled water (SDW) for 24 h. Extracts were filtered through a double layered muslin cloth followed by Whatman No. 1 filter paper and then passed through 0.22 µm micro-filter pore size to remove bacteria. Filtrates were stored at 4°C and were generally used within a week to avoid any prospective chemical alterations [30].

For organic extract's preparation, an amount of 100 g of the powdered samples (*W. somnifera* leaves, stems and fruits) was extracted by maceration with MeOH-H_2O (7:3) (v/v) for 72 h at room temperature. The hydro-methanolic extraction was carried out in triplicate. After filtration, the solvent was removed with Rotavapor under reduced pressure at 45-50°C [31]. Aqueous layer was further subjected to successive extractions using organic solvents of increasing polarity namely Chloroform (CHCl), ethyl acetate (EtOAc), and butanol (n-BuOH). CHCl, EtOAc and n-BuOH extracts were evaporated to dryness under reduced pressure at 30-40°C, 40-55°C, and 70-80°C, respectively. A sample of each dry residue (1 mg) obtained was individually dissolved in 1 ml of Dimethyl sulfoxide (DMSO). The stock extract was stored at 4°C and used within 4 days.

Determination of extraction yield

The yield (%, w/w) from all the dried extracts was calculated as:

$$Yield\ (\%) = (W1/W2) \times 100$$

where W1 is the weight of the extract after evaporation of solvent, and W2 is the weight of the plant powder.

Antifungal activity assay

The poisoned food technique [32] was used for the screening of the antifungal activity of extracts tested against FORL. Appropriate quantities of aqueous extracts from *W. somnifera* leaves, stems and fruits were added to molten PDA medium cooled to 45 to 50°C, at 1, 2, 3 and 4% (v/v) concentrations before being poured in sterilized Petri dishes (9 cm in diameter). The extracts were thoroughly mixed with the medium. DMSO and SDW were used as negative control while carbendazim-based fungicide (Bavistin ® DF) was used as positive control. After medium solidification, three agar plugs (6 mm in

Figure 1: *Withania somnifera* leaves (A), stem (B) and fruits (C).

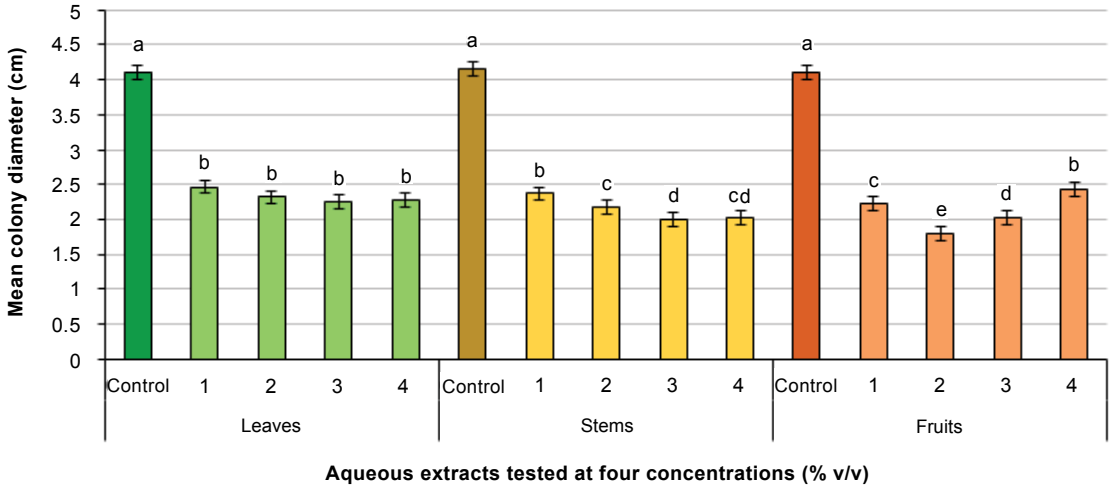

Figure 2: Mean colony diameter (cm) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* grown on PDA medium supplemented with different concentrations of aqueous extracts from *Withania somnifera* leaves, stems and fruits noted after 5 days of incubation at 25°C.
Control: Negative control (untreated control) i.e. PDA+SDW. Bars show standard error. For extracts from each organ used, bars sharing the same letter are not significantly different according to Student-Newman-Keuls (SNK) test (at $P \leq 0.05$). LSD (Organs x Concentrations tested = 0.60 cm at $P \leq 0.05$).

diameter) were removed using a pre-sterilized cork borer from 5-day-old FORL cultures and placed equidistantly in each Petri plate.

W. somnifera organic extracts were also assessed for their ability to suppress *in vitro* growth of FORL using the same method as for aqueous extracts. Dry residues from each solvent were dissolved in DMSO at the rate of 1 mg per 1 ml of DMSO. They were added to molten PDA at 1, 2, 3 and 4% (v/v) concentrations. Control PDA plates were treated using DMSO only. As done for aqueous extracts' screening, three agar plugs were placed equidistantly in each Petri plate after medium solidification.

Mean FORL colony diameter (cm) was recorded after 5 days of incubation at 25°C. Fungal growth was measured by averaging the two perpendicular diameters of each growing colony. Percentage growth inhibition (%) of FORL was calculated according to the following formula: Growth inhibition

$$Growth\ inhibition(\%) = \left[(dc - dt)/\ dc\right] \times 100$$

Where dc = Colony diameter in control plates; dt = Colony diameter in treated plates.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS 20.0 for Windows. Aqueous extracts trial was performed according to a completely randomized factorial design where *W. somnifera* organs targeted for extraction and tested concentrations represented the both fixed factors. Organic extracts trial was carried out according to a completely randomized factorial model with three factors (plant organs, type of organic extract used, and concentrations tested). Each individual treatment was replicated thrice. The whole experiment was repeated twice. Means were separated using LSD or Student-Newman-Keuls (SNK) test (at $P \leq 0.05$).

Results

Antifungal activity of *Withania somnifera* aqueous extracts

Analysis of variance revealed that FORL colony diameter varied significantly (at $P \leq 0.01$) depending on *W. somnifera* aqueous extracts used and concentrations tested; a significant interaction was detected between both fixed factors. In fact, all extracts tested were shown to be effective in suppressing FORL *in vitro* growth but with a varied degree depending on extracts' origin and concentrations used. Data given in Figure 2 showed that for leaf aqueous extract, the four concentrations used let to a significantly similar antifungal effect where FORL mycelial growth decrease was of about 42.5% relative to the untreated control. However, stem aqueous extract was found to be more active at 3 and 4% by inducing a respective reduction of pathogen growth by 52 and 51.6% compared to 43.20 and 47.60% achieved using this extract at 1 and 2%, respectively. For fruit aqueous extract, the greatest inhibition was registred at 2% concentration leading to 56.27% decrease in FORL growth as compared to the untreated control while with the other concentrations, growth inhibition ranged between 40.89 and 50.6% (Figure 4).

It should be highlighted that carbendazim-based fungicide (Bavistin ® DF, positive control) had totally (100%) suppressed pathogen growth but SDW-based treatment (negative control) did not inhibit FORL growth. All *W. somnifera* aqueous extracts tested were found to be active toward FORL with fruit aqueous extract applied at 2% being the most effective leading to 56.27% decrease in pathogen radial growth.

Antifungal activity of *Withania somnifera* organic extracts

Yields of extracts: One hundred grams of powdered *W. somnifera* leaves, stems, and fruits, individually subjected to MeOH-H_2O extraction (7:3) followed by successive extractions using CHCl, EtOAc, and n-BuOH yielded variable dry residues depending on organs used. In fact, leaf extracts' yields were of about 0.99, 0.84, and 1.38% for chloroform, ethyl acetate and butanol fractions, respectively, compared to 0.95, 0.93, and 1.23% yielded from stems and to 0.91, 1.18, and 1.53% obtained from fruits' fractions (Table 1).

Antifungal activity

ANOVA analysis revealed that FORL colony diameter, noted after 5 days of incubation at 25°C, varied significantly depending on organs used for extraction, organic extracts tested and concentrations used. A significant interaction (at $P \leq 0.01$) between the three fixed factors was also detected.

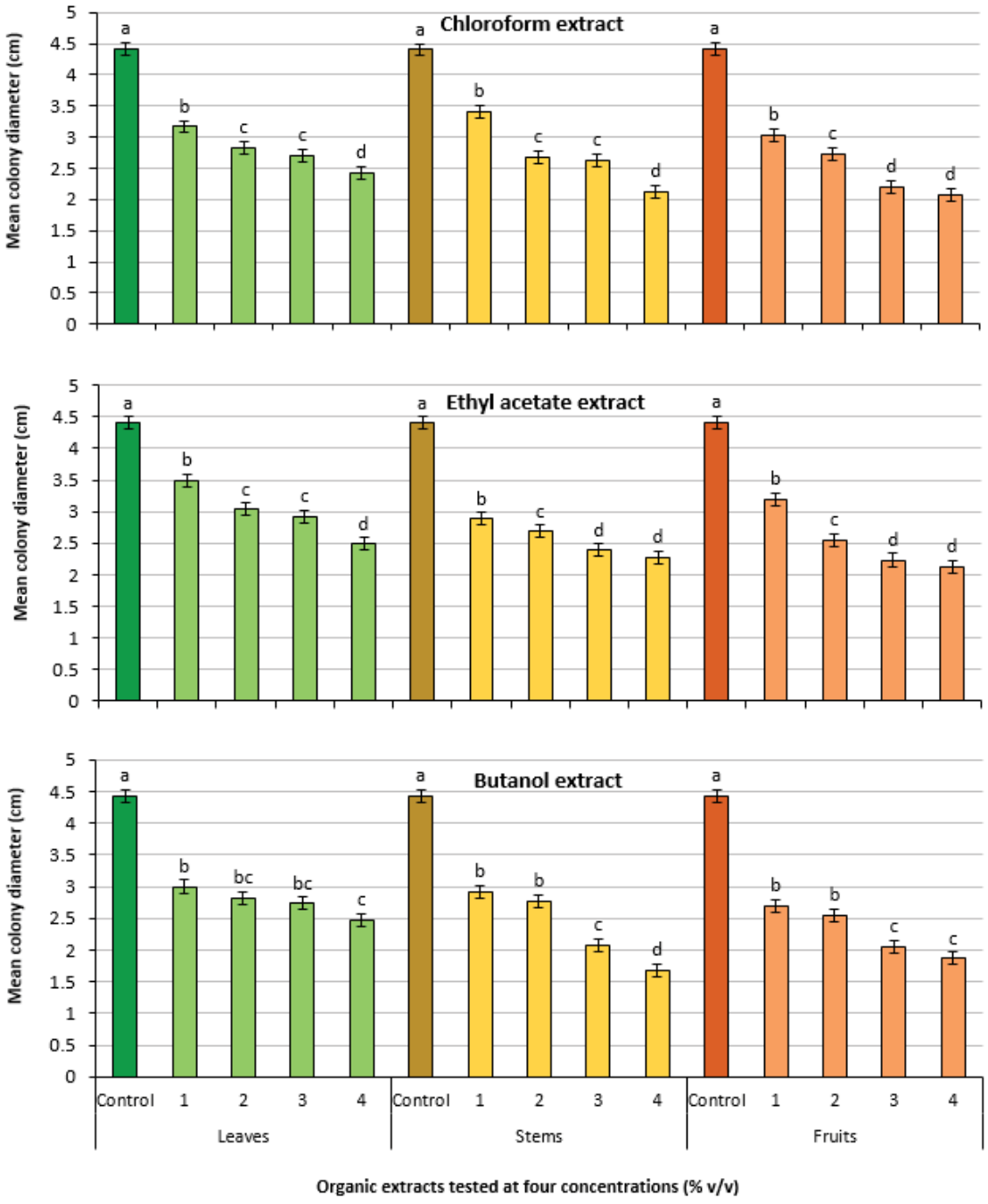

Figure 3: Mean colony diameter of *Fusarium oxysporum* f. sp. *radicis-lycopersici* colonies grown on PDA medium supplemented with different concentrations of *Withania somnifera* leaf, stem and fruit organic extracts noted after 5 days of incubation at 25°C.
Negative Control: PDA + DMSO (untreated control). Bars show standard error. For extract type from each organ used, bars sharing affected by the same letter are not significantly different according to Student-Newman-Keuls (SNK) test (at $P \leq 0.05$). LSD (Organs x Type of extracts x Concentrations tested = 0.41cm at $P \leq 0.05$).

Data given in Figure 3 showed that all organic extracts tested exhibited antifungal activity and had inhibited pathogen radial growth, as compared to the untreated control, in a concentration-dependant manner. In fact, pathogen inhibition reached 45.28, 51.89, and 53.21% using *W. somnifera* chloroform extracts from leaves, stems, and fruits used at 4%, respectively, compared to 28.30, 22.34, and 31.32% achieved using these extracts at 1% (Figure 3).

For ethyl acetate fractions, FORL radial growth was 43.56, 48.48, and 51.70% lesser than the untreated control when grown on PDA supplemented at 4% with leaf, stem and fruit ethyl acetate fractions in contrast to 20.45, 34.47, and 27.55% noted when these extracts were tested at 1% (Figure 3).

Butanolic extracts from *W. somnifera* leaves, stems and fruits, used at 4%, had also suppressed pathogen growth by 44.15, 62.03,

	Start material (g)	Yield (%)		
Samples		**Chloroform**	**Ethyl acetate**	**Butanol**
Leaf powder	100	0.99	0.84	1.38
Stem powder	100	0.95	0.93	1.23
Fruit powder	100	0.91	1.18	1.53

Table 1: The percentage yields of organic extracts prepared from *Withania somnifera* leaves, stems and fruits.

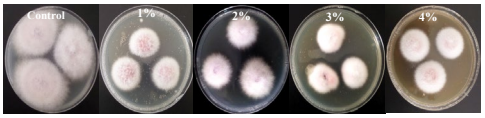

Figure 4: *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) colonies grown on PDA medium supplemented with aqueous extracts from *Withania somnifera* fruits, tested at four concentrations (1, 2, 3, and 4% v/v) recorded after 5 days of incubation at 25°C.
Negative Control: Untreated control (PDA + distilled water). The highest inhibition (56.28%) of FORL mycelial growth was recorded at the concentration 2% as compared to the untreated control.

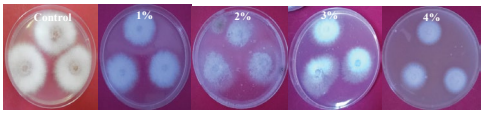

Figure 5: *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) colonies grown on PDA medium supplemented with butanolic extract from *Withania somnifera* fruits tested at four concentrations (1, 2, 3, and 4% v/v) recorded after 5 days of incubation at 25°C.
Negative Control: Untreated control (PDA + DMSO). The highest inhibition (57.78%) of FORL mycelial growth was recorded at the concentration 4% as compared to the untreated control.

and 57.74%, relative to control, respectively, whereas their antifungal potential decreased to 32.08, 34.21, and 38.87% when applied at 1% (Figures 3 and 5).

The present screening showed that *W. somnifera* fruit extracts were more active against FORL than those from leaves and stems. Similarly, butanolic extract was found to be relatively more effective in suppressing FORL growth than chloroform and ethyl acetate extracts which showed similar effect. In addition, for concentrations used, it was shown that highest mycelial growth inhibition of the targeted pathogen was mainly reached at the highest concentration applied (4%) (Figures 3 and 6).

It should be highlighted that carbendazim-based fungicide (Bavistin ® DF, positive control) had totally (100%) suppressed pathogen growth but DMSO-based treatment (negative control) did not inhibit FORL growth.

Discussion

The search of new fungicides effective, biodegradable and with greater selectivity is necessary to face chemicals' related problems. Natural plant-derived products are safe and could be integrated into pest managment programs as they display antifungal activity without being phytotoxic [33]. In this study, aqueous, chloroform, ethyl acetate, and butanol extracts from *W. somnifera* leaves, stems and fruits, were screened *in vitro* at four concentrations (1, 2, 3, and 4%) for their ability to suppress FORL growth.

The use of solvents with varying polarity in the extraction procedure is determinal for the successful isolation of compounds with different ranges of polarity. Results showed that butanol extraction

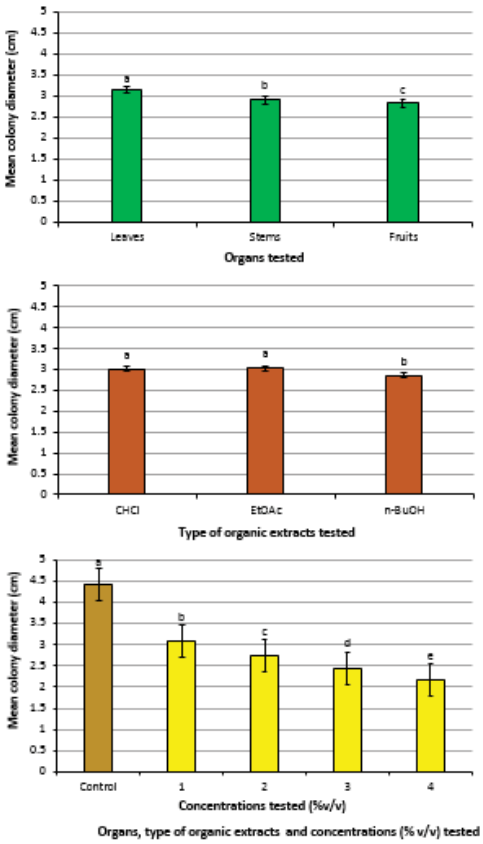

Figure 6: Mean colony diameter (cm) of *Fusarium oxysporum* f. sp. *radicis-lycopersici* grown on PDA medium supplemented with different organic extracts from *Withania somnifera* depending on organs used, concentrations tested and organic solvents used for extraction noted after 5 days of incubation at 25°C.
Negative control (untreated control): PDA+DMSO. Leaves; Stems; Fruits: Organs used from *Withania somnifera*. CHCl: Chloroform; EtOAc: Ethyl acetate; n-BuOH: Butanol. 1; 2; 3 and 4 (% v/v): Concentrations used for each treatment. Bars show standard error. For each organ, for each type of organic extract used, and for each concentration tested, bars sharing the same letter are not significantly different according to Student-Newman-Keuls (SNK) test (at $P \leq 0.05$).

yielded more dry residues than that of chloroform and ethyl acetate. In fact, butanol extractions' yields were 1.38, 1.23, and 1.53% for fractions from leaves, stems and fruits, respectively. It was also shown that butanol stems extracts induced the highest mycelial growth inhibition (62.03%) compared to chloroform (51.89%) and ethyl acetate (48.48%) fractions. This antifungal potential may be due to the presence of some polar constituents such as glycoside, saponins, tannins, and flavonoides which could be present in butanolic fractions as shown in previous studies [34].

All *W. somnifera* aqueous extracts had significantly reduced FORL mycelial growth at all concentrations tested. Fruit extract was found to be the most active at 2% leading 56.28% lower growth relative to the untreated control. However, for the other organs, the highest antifungal potential was noted at 4% (40.08 and 43.20% achieved using leaf and stem extracts, respectively). Similarly, Shafique et al. [35] showed that *W. somnifera* aqueous extracts had significantly reduced by 49% the incidence of the black pointed disease of *Triticum aestivum* incited by *A. alternata*. This significant antifungal effect could probably be

attributed to some bioactive polar and water soluble metabolites. Indeed, phytochemical analysis of *W. somnifera* aqueous leaf and stem extracts revealed the presence of carbohydrates, glycosides, alkaloids, phytosterols, fixed oils, phenolic compounds, flavonoids, withaferin A, ascorbic acid, anthocyanin, and polyphenols which are known to have antifungal potential [34,36-38]. However, few data are available concerning use of wild *Solanaceae* metabolites for FCRR control in tomato. However, Shivpuri and Gupta [39] showed that leaf aqueous extracts from *W. somnifera*, *Datura stramonium* and *Physalis longifolia* are able to inhibit *Sclerotinia sclerotiorum*. On other hand, Onalar and Yilar [40] showed that flower aqueous extracts from *Trachystemon orientalis* L. have completely inhibited FORL mycelial growth when applied at the concentrations 5, 7, 10, and 20%. In addition, Marley [41] showed that dry neem (*Azadirachta indica*) seed extract has totally suppressed *F. oxysporum* growth at all tested concentrations (10, 20 and 30%).

Chloroform, ethyl acetate and butanolic extracts showed significant antifungal potential against FORL mycelial growth but with varied levels depending on types of organic extracts, plant material used for extraction, and concentrations tested. These results are in agreement with findings from previous studies. Indeed, Khrishnamoorthy et al. [42] demonstrated that antifungal activity is pathogen-specific and depends on solvent, crude extract concentration, temperature, and plant parts used for extraction of secondary metabolites. This could be attributed to their differences in chemical nature, polarity, and solubility of active biomolecules in each used solvent. Few studies have reported the antifungal activity of *W. somnifera* organic extracts (chloroform, ethyle acetate, and butanolic) against FORL. In fact, Khan and Nasreen [43] showed that *W. somnifera* methanolic extracts reduce the colony diameter of *F. oxysporum* by 71.11% as compared to the untreated control. It was also shown that *W. somnifera* protein fractions exhibit strong antifungal activity.

For chloroform extracts, those from stems and fruits were found to be more effective against FORL where fungus radial growth was reduced by more than 50% relative to 45% obtained using leaf extracts. Similarly, chloroform extract from *W. somnifera* calyx shows highest antibacterial activity toward *B. subtilis* [44]. Our results are also in accordance with those of Javaid and Munir [45] who showed that *W. somnifera* fruit and leaf methanolic extracts are highly effective against *Ascochyta rabiei*, the causal of chickpea blight disease, leading to 41 and 43% decrease in fungal biomass, respectively.

Ethyl acetate extracts from *W. somnifera* leaves, stems, and fruits had significantly inhibited the growth of the target fungus by more than 45%. Ethyl acetate fruit extract had limited pathogen growth by 51.70% at the highest concentration tested (4%). Similarly, Uddin et al. [46] demonstrated that ethyl acetate fraction from *W. somnifera* fruits was found to be active againt various bacterial agents such as *Klebsiella pneumoniae*, *S. aureus*, and *B. subtilis*. In this regard, Uddin et al. [46] reported that preliminary phytochemical screening of *W. somnifera* fruits revealed the presence of bioactive secondary metabolites such as alkaloids, saponins, glycosides, steroids, terpenoids, tannins, coumarins, and reducing sugars which are reported to have antimicrobial properties.

The present study pointed out that FORL was more sensitive to *W. somnifera* butanolic fractions compared to chloroform and ethyl acetate ones. The strongest inhibition, of about 62%, occurred using stem butanolic extract applied at 4%. Girish et al. [8] identified a monomeric glycoprotein from *W. somnifera* roots with potent activity against bacteria and phytopathogenic fungi. Some important chemical compounds are identified in *W. somnifera* including withaferin A, flavonol, glycosides, glycowithanoldes, phenolics, and sterols which could be involved in its antifungal activity.

Several works reported the antifungal effects of *W. somnifera* organic extracts against some phytopathogenic fungi. Indeed, Krishnamurthy et al. [47] showed that methanolic leaf powder of *W. somnifera* inhibited, by about 51%, the growth of *A. flavus* in soybean seeds till six months of storage. Similarly, Jat and Agalave [48] found that *Penicillium notatum* and *P. chrysogenum* growth was hampered by the leaf extract of *W. somnifera*. In addition, Javaid and Akhtar [49] showed that methanolic extracts from *W. somnifera* roots decreased the fungal biomass of *F. oxysporum* f. sp. *cepae* by up to 93%.

Conclusion

Current work is the first report on the *in vitro* antifungal activity of chloroform, ethyl acetate and butanol extracts from *W. somnifera* leaves, stems and fruits, against FORL. Results showed that *W. somnifera* extracts exhibited significant antifungal effect against this pathogen. Stem and fruit butanol extracts were found to be the most bioactive, mainly when used at the highest concentration (4%). Bioactive compounds from *W. somnifera* extracts, involved in the registered antifingal activity, could serve as a potential source of natural derived fungicides once their efficacy proved *in vivo* on FORL-infected tomato plants. Moreover, purification and chemical identification of their bioactive compounds may elucidate more accurately the mechanisms of action involved in pathogen inhibition.

Acknowledgments

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