Revolutionizing Agriculture: Synergistic Antifungal Activity of Medicinal Plant Extracts against soil-borne phytopathogenic fungi

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Research Article

Keywords: Plant extracts, mycelial growth, pathogenic fungi

Posted Date: December 17th, 2024

DOI: https://doi.org/10.21203/rs.3.rs-5320837/v1

Additional Declarations: No competing interests reported.

Abstract

A study was conducted to assess the effectiveness of various natural plant extracts on soil fungi, particularly targeting phytopathogenic and mycotoxigenic species *Alternaria alternata, Fusarium oxysporum, Rhizoctonia solani, Cladosporium cladosporioides, Penicillium citrinum, Curvularia lunata, Fusarium metavorans, Fusarium solani* and *Aspergillus aflatoxiformans*, were used in the present study. In vitro studies were carried out to evaluate the antifungal activity of three plant extracts performed both in aqueous and methanolic solutions. The findings indicated that the plant extracts exhibited robust antifungal activity, resulting in significant inhibition of mycelial growth across all tested fungi compared to the control. The extracts from *Phytolacca acinosa* and *Atropa acuminata* demonstrated the highest effectiveness in inhibiting the mycelial growth of the tested fungi, whereas *Salix alba*exhibited comparatively lower efficacy. The findings of this study validate the potential of plant extracts as natural fungicides for controlling pathogenic fungi, there by reducing dependence on synthetic fungicides. However, the extracts from *Phytolacca acinosa* and *Atropa acuminata*, identified as the most efficient, hold promise as potential agents for controlling these pathogenic fungi.

Introduction

Natural products of some plants have been used to control plant disease in order to avoid the hazardous effects of chemicals (Rahber-Bhatti, 1988; Bowers and Locke, 2000; Momin *et al.*, 2001). Recent reports have highlighted advancements in developing safer anti-fungal agents, including plant extracts, for controlling phytopathogens in agriculture (Imtiaj et al., 2005; Tumen et al., 2013). Essential oils and their components have demonstrated efficacy as antifungal agents. (Daferera et al., 2000; Sridhar et al., 2003). Several studies have documented the fungicidal properties of neem oil (Kazmi et al., 1995). According to Locke (1995), field experiments revealed complete control of *Alternaria alternata, Aspergillus niger*, and *Fusarium oxysporum* with 2–10% neem oil application. Mustard seed oil has also been observed to exhibit antifungal activity. (Dhingra et al., 2004). Houghton et al. (2006) documented the antifungal activity of *asafoetida* against *Microsporeum gypseum* and *Trichophyton interdigitale*. Thyagaraia & Hosono (1996) also studied the inhibition effect of asafoetida on *Fusarium solani, Rhizopus sporus, Penicillium commune* and *Mucor dimorphosphorous*. Extracts from various plants, including garlic (*Allium sativum*) (Obagwu and Korsten, 2003), *Azadirachta indica, Moringa oleifera* (Adandonon et al., 2006), *Ferula communis, Dittrichia viscose*, and *Juniperus communis*, have been studied for their potential applications. Menghani and Sharma (2012) have conducted tests on many other soil-borne fungi. Alkhail (2005) demonstrated significant biological activity of extracts from *Allium sativum*, *Azadirachta indica*, and *Eugenia caryophyllus* against fungi such as *F. oxysporum* and *Botrytis cinerea*.

The antimicrobial and antitoxin properties of certain plants, herbs, and their constituents have been recorded since the late 19th century (Saadabi, 2006; Fawzi et al., 2009; Zaker and Mosallanejad, 2010; Abdulghaffar et al., 2010; Abdel Ghany and Hakamy, 2014). These natural plants include alfalfa, black seed, basil, datura, acacia, lemon grass, garlic, a triplex, neem, ginger, *Juniperus procera*, and eucalyptus

(Omar and Abd-El-Halim, 1992; Aly et al., 2000; Aly and Bafiel, 2008; Abdel Ghany 2014). These natural alternatives are considered safer for humans and the ecosystem compared to chemical antifungal compounds, can be utilized by the public for thousands of years to enhance the flavor and aroma of foods, as well as their economic value (Shelef, 1983). In recent times, a considerable number of synthetic fungicides have faced bans in the Western world due to their elevated and acute toxicity, prolonged degradation periods, accumulation in the food chain, and other related concerns (Satish *et al*., 1999). However, an emerging alternative to haphazard chemical synthesis is the exploitation and utilization of naturally occurring products possessing fungicidal properties. Plants produce a vast array of secondary metabolites, and it is widely believed that a important portion of this chemical diversity functions to safeguard plants against plant pathogens (Negri, M., *et al*.,). Numerous biocontrol fungi and extracts derived from various medicinal plants have gained significant popularity and scientific attention due to their antifungal and antibacterial properties (Santas et al., 2010; Parveen et al., 2016a; Koka et al., 2017). They are considered to be less hazardous compared to chemical fungicides, making them a viable alternative for controlling fungal rot diseases. (Jobling, 2000). Employing these plant extracts to combat fungal diseases marks a crucial endeavor in evaluating the extent of diversity within the varied natural flora (Khandare and Vasait, 2017). Therefore, aim of the present study in an approach towards ecofriendly management strategy, extracts of three different medicinal plants, viz. *Salix alba* L., *Phytolacca acinosa* Roxb., and *Atropa acuminata* Royle ex Lindl. were evaluate for their antifungal activity against some phytopathogenic fungi.

Materials and Methods

The study was conducted in the mycology and plant pathology, Bio-Control Lab, Department of Botany University of Kashmir, Srinagar, India.

Plant collection and identification

During the present study fresh plant material of three medicinal plants, viz. *Salix alba* L., *Phytolacca acinosa* Roxb*.,* and *Atropa acuminata* Royle ex Lindl were collected from the different study area of Kashmir Himalaya, India. *Salix alba* was collected from the Kashmir University Botanical Garden, *Phytolacca acinosa* from Doodhpathri, and *Atropa acuminata* from Gulmarg. All specimens were identified by Akhtar H. Malik, with voucher IDs KASH-242, KASH-243, and KASH-244, respectively. The authenticity of the plants were confirmed in Plant Taxonomy and also voucher specimen of all plants has been deposited in the herbarium of Department of Botany University of Kashmir, Srinagar.This process is commonly undertaken in scientific research to provide a reference for future studies and to ensure the accuracy of the identified plant specimens.

Preparation of plant extracts

Healthy leaves from three medicinal plants were thoroughly rinsed with running tap water to remove any soil and debris. They were then shade-dried at room temperature (25 ± 2°C) for 15 days in the laboratory. After drying, the leaves were ground into a fine powder and stored in airtight containers. Approximately

20g of coarsely powdered leaves (20g/100mL) were extracted separately using a Soxhlet extractor for 8 to 10 hours at (30-50°C) first with methanol and then with water, to isolate non-polar and polar compounds, respectively.

Fungal pathogens

Rhizoctonia solani, Alternaria alternata, Fusarium oxysporum, *Cladosporium cladosporioides, Penicillium citrinum, Curvularia lunata, Fusarium metavorans, Fusarium solani* and *Aspergillus aflatoxiformans* isolated pure cultures of fungal strains *were used in the present study.* Test fungal culture stocks were preserved at 4 °C on potato dextrose agar (PDA) plates.

Impact of selected plant extracts on pathogenic fungal mycelial growth

The food poisoning technique was used to examine the antimycotic activity of extraction of *Salix alba, Phytolacca acinosa*, and *Atropa acuminata* on the development of mycelial colonies of soil pathogenic fungi (Falck, 1907; Grower and Moore, 1962). A suitable amount of methanol and double-distilled water were added to the reference concentration, and the mixture was then mixed with Potato Dextrose Agar medium to create the various quantities, S/1, S/2, and S/3. 15–20 ml of the solution were placed in sterilised Petri dishes and left to harden in a laminar airflow. A 5 mm mycelial disc of the pathogenic fungus was placed in the centre of the petri plates after they had solidified, and they were then incubated for 4–7 days at 25±2°C. The percentage of growth inhibition brought on by different treatments at different doses was computed using the following formula:

$$\text{Percentage inhibition (\%)} = \frac{dc-dt}{dc} \times 100$$

Where “dc” denotes the average diameter of the fungal colony in control and “dt” denotes the average diameter of the fungal colony.

Statistical analysis

The outcomes for each experiment are displayed as mean ± SD (standard deviation). Parallel studies were done to assess each of the experiments listed and compared in each table, and different statistical analyses were run for each test. SPSS 23 (SPSS Inc., Chicago, IL, USA) was used to analyze variance (ANOVA) with Duncan multiple comparison test for the antifungal efficacy of plant extracts against pathogenic soil fungi.

Results

Evaluation of botanicals for the management of pathogenic soil fungi

During this study, different concentrations of extracts of some medicinal plants, namely *Salix alba, Phytolacca acinosa*, and *Atropa acuminata* were tested for their efficacy on mycelial growth and reduction in germination of spores of some pathogenic soil fungi, in order to evaluate their antimycotic activity.

Phytochemical screening of plant extracts of tested plants

The presence of several phytochemicals such as phenols, alkaloids, tannins, flavonoids, terpenoids, etc. was assessed in the aqueous and methanolic extracts of the medicinal plants, viz. *Salix alba, Phytolacca acinosa* and *Atropa acuminata* (Table 1) in order to observe their effective phytochemicals. The aqueous and methanolic extracts of *Salix alba, Phytolacca acinosa*, and *Atropa acuminata* all contained the phytochemicals, and it was observed that they were more efficient against various fungal pathogens. The aqueous and methanolic extracts of *Phytolacca acinosa* were found to be devoid of alkaloids and terpenoids. These plants were also tested for their antifungal potential against some isolated soil pathogens, such as *Alternaria alternata, Fusarium oxysporum, Rhizoctonia solani, Cladosporium cladosporioides, Penicillium citrinum, Curvularia lunata, Fusarium metavorans, Fusarium solani* and *Aspergillus aflatoxiformans.*

Efficacy of plant extracts on pathogenic soil fungi using food poisoning technique

Mycelial growth inhibition was used to assess the antifungal effects of both aqueous and methanolic extracts of some medicinal plants such as *Salix alba, Phytolacca acinosa*, and *Atropa acuminata* on soil pathogenic fungi. All of the aqueous and methanolic plant extracts considerably slowed the mycelial growth of all of the tested fungal pathogens. However, it was shown that methanolic plant extracts were more efficient than aqueous plant extracts.

Efficacy of various concentrations of plant extracts on the mycelial growth inhibition of *Alternaria alternata* (Fr.) Keissler

In the current investigation, it was observed (Table 2, Fig.1) that all of the tested plant extracts at different concentrations strongly reduced mycelial growth of *Alternaria alternata*. However, the lower concentrations of both types of plant extracts showed least efficacy. It has been observed that methanolic plant extracts was more efficient than aqueous plant extracts. Both plant extracts of were found more effective, with the highest reduction in mycelial growth *Alternaria alternata* was caused by both forms of phytoextracts (methanolic and aqueous) of *Atropa acuminata Phytolacca acinosa* and S*alix alba.* The inhibition of mycelial growth by methanolic and aqueous plant extracts of *Atropa acuminate* against *Alternaria alternata* varied between 4.66 mm to 11.00 mm and 11.66 mm to 17.33 mm in different concentrations of plant extracts, respectively. However, both solvent (methanolic and aqueous) phytoextracts of *Phytolacca acinosa* varied reduction in mycelial growth from 5.33 mm and 12.66 mm and 14.00 mm to 19.00 mm, respectively, mycelial growth reduction by various concentrations of methanolic and aqueous phytoextracts of *Salix* varied from 6.66 mm to 12.66 mm and 14.33 mm to 21.33 mm, respectively.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Fusarium oxysporum* (Schl.) emend. Snyder and Hansen

The current investigation indicated that the application of varying quantities of phytoextracts significantly reduced the fungal mycelial growth of *Fusarium oxysporum* (Table 3, Fig. 2). Methanolic plant extracts were shown to be more effective than aqueous plant extracts. Compared to the lower dosages, the higher values demonstrated greater efficacy. *Phytolacca acinosaca* plant extracts, both methanolic and aqueous, inhibited mycelial growth against *Fusarium oxysporum* to the greatest extent. *Atropa acuminata* and *Salix alba* plant extracts came next. At various concentrations of plant extracts, the suppression of mycelial growth by *Phytolacca acinosa* methanolic and aqueous plant extracts against *Fusarium oxysporum* ranged from 4.33 mm to 13.66 mm and 8.33 mm to 16.66 mm, respectively. In contrast, the mycelial growth inhibition in *Atropa acuminata* methanolic and aqueous plant extracts ranged from 4.66 mm to 15.66 mm and 10.33 mm to 18.66 mm, respectively. At varying doses, the inhibition of mycelial growth caused by methanolic and aqueous plant extracts of *Salix alba* ranges from 5.00 mm to 16.33 mm and 17.00 mm to 20.00 mm, respectively.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Rhizoctonia solani* Kuhn

In the current investigation, it was revealed (Table 4, Fig.3) that all of the tested plant extracts caused considerable reduction in mycelial growth of *Rhizoctonia solani* at different concentrations. It has been observed that methanolic plant extracts proved efficient than aqueous plant extracts. The higher concentrations proved more effective than lower concentrations. Both methanolic and aqueous plant extracts of *Atropa acuminata* caused higher mycelial growth inhibition against *Rhizoctonia solani* followed by plant extracts of *Salix alba and Phytolacca acinosa.* The inhibition of mycelial growth in different concentrations of methanolic and aqueous plant extracts of *Atropa acuminata* against *Rhizoctonia solani* varied between 5.00 mm to 11.00 mm and 31.00 mm to 39.00 mm respectively. Likewise, the inhibition of mycelial growth in methanolic and aqueous plant extracts of *Salix alba* varied 5.33 mm and 12.00 mm and 33.66 mm to 44.00 mm, respectively. Inhibition in mycelial growth due to methanolic and aqueous plant extracts of *Phytolacca acinosa* at different concentrations varied between 5.66 mm to 14.00 mm and 41.33 mm to 46.00 mm, respectively.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Cladosporium cladosporioides* (Fresen.) G. A. de Vries

In the present study, it was revealed (Table 5, Fig.4) that all of the tested plant extracts reduced *Cladosporium cladosporioides* mycelial growth at their different concentrations. It was further observed that the aqueous phytoextracts showed least efficacy in comparison to methanolic phytoextracts. Reduction in mycelial growth of *cladosporium cladosporioides* was highest treatment with both methanolic and aqueous extracts *Phytolacca acinosa* of followed *Atropa acuminate and Salix alba at the equal concentrations.* The inhibition of mycelial growth by methanolic and aqueous plant extracts of *Phytolacca acinosa* against *Cladosporium cladosporioides* varied between 4.00 mm to 14.00 mm and

10.00 mm to 18.00 mm in different concentrations of plant extracts, respectively. However, the inhibition of mycelial growth in methanolic and aqueous plant extracts of *Atropa acuminata* varied 4.66 mm and 16.66 mm and 11.33 mm to 19.00 mm, respectively. Inhibition in mycelial growth due to methanolic and aqueous plant extracts of *Salix alba* at different concentrations varied between 8.33 mm to 19.33 mm and 15.66 mm to 23.33 mm, respectively.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Penicillium citrinum* Thom

In the present study, it was revealed (Table 6, Fig.5) that all of the tested plant extracts caused *Penicillium citrinum* mycelial growth inhibition at different concentrations. The methanolic plant extracts proved efficient than aqueous plant extracts. The higher concentrations of plant extracts proved more efficient than lower concentrations. Both methanolic and aqueous plant extracts of *Phytolacca acinosa* caused maximum mycelial growth inhibition of *Penicillium citrinum* followed by plant extracts of *Atropa acuminata and Salix alba.* The inhibition of mycelial growth by methanolic and aqueous plant extracts of *Phytolacca acinosa* against *Penicillium citrinum* varied between 4.33 mm to 13.33 mm and 13.33 mm to 19.00 mm at different concentrations respectively. However, the inhibition of mycelial growth in methanolic and aqueous plant extracts of *Atropa acuminata* varied 5.00 mm and 15.66 mm and 13.00 mm to 20.66 mm, respectively. Inhibition in mycelial growth due to methanolic and aqueous plant extracts of *Salix alba* at different concentrations varied between 6.00 mm to 16.00 mm and 14.00 mm to 18.33 mm, respectively. The lower concentration caused reduction in mycelial growth but to lesser extent.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Curvularia lunata* Boedijn

The results revealed that various on concentrations phytoextracts decreased mycelial growth development of fungus *Curvularia lunata.* The methanolic the aqueous phytoextracts showed least efficacy in comparison to methanolic extracts. Both methanolic and aqueous plant extracts of *Atropa acuminate extracts* caused highest inhibition in *Curvularia lunata* mycelial development followed by *Salix alba and Phytolacca acinosa.* The inhibition of mycelial growth by methanolic and aqueous plant extracts of *Atropa acuminata* against *Curvularia lunata* varied between 6.00 mm to 14.00 mm and 10.00 mm to 14.00 mm at different concentrations of plant extracts, respectively. However, the inhibition of mycelial growth in methanolic and aqueous plant extracts of *Salix alba* varied 8.00 mm and 15.00 mm and 12.33 mm to 20.66 mm, respectively. Inhibition in mycelial growth due to methanolic and aqueous plant extracts of *Phytolacca acinosa* at different concentrations varied between 8.33 mm to 18.00 mm and 13.00 mm to 24.00 mm, respectively. The lower concentration caused reduction in mycelial growth but to lesser extent (Table 7, Fig. 6).

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Fusarium metavorans* Al-Hatmi S.A. Ahmed and de Hoog

At varying concentrations, the current investigation found that all of the tested plant extracts significantly inhibited the mycelial growth of *Fusarium metavorans* (Table 8, Fig. 7). Compared to aqueous plant extracts, methanolic plant extracts demonstrated superior efficiency. Compared to lesser dosages, the higher amounts were more effective. *Salix alba* plant extracts, both methanolic and aqueous, were shown to be more efficient than those of *Atropa acuminata* and *Phytolacca acinosa*, respectively, with the greatest mycelial growth suppression against *Fusarium metavorans*. At varying doses of plant extracts, the mycelial growth inhibition of *Salix alba* methanolic and aqueous plant extracts against *Fusarium metavorans* ranged from 5.00 mm to 11.33 mm and 6.00 mm to 16.66 mm, respectively. However, the range of mycelial growth suppression by *Atropa acuminata* methanolic and aqueous plant extracts was 7.00 mm, 13.00 mm, 8.00 mm, and 18.33 mm, respectively. Mycelial growth inhibition caused by *Phytolacca acinosa* methanolic and aqueous plant extracts at varying doses ranged from 7.00 mm to 13.33 mm and 7.66 mm to 19.66 mm, respectively. To a lesser degree, mycelial growth was also inhibited by the reduced concentration.

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Fusarium solani* Snyder and Hansen

In the resent study, it was observed (Table 9, Fig. 8) that all of the tested plant extracts caused reduction in *Fusarium solani* mycelial growth at their different concentrations. The phytoextracts prepared in methanol were more effective in comparison to aqueous phytoextracts. Lower concentrations of all phytoextracts were tested to be least effective as compared to higher concentrations. Both methanolic and aqueous plant extracts of *Atropa acuminata* were highly effective, and caused maximum mycelial growth inhibition against *Fusarium solani* followed by plant extracts of *Phytolacca acinosa* and *Salix alba.* The inhibition of mycelial growth by methanolic and aqueous plant extracts of *Atropa acuminata* against *Fusarium solani* varied between 7.00 mm to 18.66 mm and 12.33 mm to 24.00 mm at different concentrations of plant extracts, respectively. However, the inhibition of mycelial growth by methanolic and aqueous plant extracts of *Phytolacca acinosa* varied 9.33 mm and 19.00 mm and 13.00 mm to 25.00 mm, respectively. Inhibition in mycelial growth due to methanolic and aqueous plant extracts of *Salix alba* at different concentrations varied between 11.66 mm to 25.33 mm and 15.00 mm to 27.33 mm, respectively. The lower concentration also caused reduction in mycelial growth but to a lesser extent.

Efficacy of different concentrations of selected phytoextracts on the inhibition of mycelial growth in *Aspergillus aflatoxiformans* Frisvad, Ezekiel, Samson, and Houbraken

The results of this study (Table 10, Fig. 9) showed that all tested plant extracts reduced mycelial growth of *Aspergillus aflatoxiformans* at various concentrations. Methanolic extracts demonstrated greater efficacy than aqueous extracts, and higher concentrations were more effective than lower ones. Both methanolic and aqueous extracts of *Phytolacca acinosa* showed the highest efficacy in inhibiting the mycelial growth of *Aspergillus aflatoxiformans*, followed by extracts from *Salix alba* and *Atropa acuminate*. The mycelial growth inhibition of *Aspergillus aflatoxiformans* by methanolic extracts of

Phytolacca acinosa ranged from 7.66 mm to 15.00 mm, while the aqueous extracts inhibited growth from 9.00 mm to 18.00 mm, depending on the concentration used. The inhibition of mycelial growth by methanolic and aqueous extracts *of Salix alba* ranged from 8.00 mm to 16.00 mm and 8.33 mm to 18.33 mm, respectively. For *Atropa acuminata,* methanolic extracts inhibited growth between 9.00 mm and 17.00 mm, while aqueous extracts showed inhibition from 10.00 mm to 18.66 mm at various concentrations. Lower concentrations also reduced mycelial growth, though to a lesser extent.

Discussion

The antifungal activity of aqueous and methanolic extracts of three different medicinal plants, viz. *Salix alba, Phytolacca acinosa*, and *Atropa acuminata* at various concentrations was tested against a few selected soil fungi such as *Alternaria alternata, Fusarium oxysporum, Rhizoctonia solani, Cladosporium cladosporioides, Penicillium citrinum, Curvularia lunata, Fusarium metavorans, Fusarium solani* and *Aspergillus aflatoxiformans*. The effects of these plant extracts on mycelial growth inhibition and spore germination inhibition were assessed in order to evaluate their antimycotic activity. All aqueous and methanolic extracts of test plants considerably suppressed the mycelial growth of all of the test fungal pathogens. However, it was shown that methanolic plant extracts were more efficient than aqueous plant extracts in preventing the mycelium growth and spore germination of soil pathogens. The plant extracts can potentially be used for antifungal activity against soil pathogenic fungi. Similarly plant extracts inhibited the growth of some phytopathogenic fungi as reported by Taskeen-Un-Nisa *et al*., 2010; Znini et al., 2011; Raji and Raveendran, 2013; Parveen et al., 2017; Koka et al., 2017 whose results are in comparison with our results Satish et al. (2007) and Paola et al. (2011) reported the antimycotic effect of aqueous extracts of fifty-two plants against *Aspergilluscandidus*, *Aspergilluscolumnaris*, *Aspergillus flaviceps, Aspergillus flavus*, *A. fumigatus, A. niger, A. ochraceus* and *Aspergillus tamari.* And the extracts of *C. scolymus*, *S. sclarea*, *S. officinalis*, and *Lippia alba* were similarly more effective than the other plant extracts in inhibiting mycelial growth. Similarly, Sesan et al. (2015) examined the antimycotic effects of nine plant extracts, viz. *Achillea millefolium, Allium sativum, Artemisia dracunculus, Hyssopus officinalis, Mentha sp. Rosmarinus officinalis, Satureja hortensis, Tagetes patula* and *Valeriana officinalis* against *Botrytis cinerea*. Similarly many workers like Ramaiah and Garampalli (2015) found the antifungal activity of *Azadirachta indica, Oxalis latifolia Solanum indicum,and* against *Fusarium oxysporum;* Buch and Arya (2015) showed that plant extracts had antifungal properties against the fungi *Fusarium oxysporum*, *Alternaria alternata*, and *Chaetomium globosum* and Gwa et al. (2018) obtained the antifungal activity of *Piper nigrum Nicotiana tabacum*, *Zingiber officinale*, *Azadirachta indica*, and *Carica papaya* against *Penicillium expansum*. Their findings showed that *P. nigrum* and *Z. officinale* were the two plants that were most inhibited the growth of pathogens. However, *C. papaya* was least competent plant extract in controlling the growth of the pathogen. Furthermore, the higher concentrations proved effective than lower concentrations. Koka et al. (2019) reported the antifungal efficacy of aqueous extracts of *Ajuga bracteosa, Taraxacum officinale*, *Mentha arvensis* and *Iris kashmiriana* against *Trichothecium roseum*. These plant extracts contain many secondary metabolic products and alkaloids which inhibit the fungal growth (Kaur and Arora, 2015). Hence these plant

extracts can be further explore for analysis of the bioactive compounds which can be processed for forming of plant products to be incorporated in IPM as an alternative disease management strategy.

Conclusion

This study showed that methanolic and aqueous extracts of the medicinal plants, *Salix alba, Phytolacca acinosa and Atropa acuminata* caused significant reduction on mycelial growth of the isolated soil fungal pathogens. Amongst the tested plant extracts, Phytoextracts obtained from *Phytolacca acinosa and Atropa acuminata* reduced mycelial growth of maximum isolated soil fungal pathogens, while as *Salix alba* was found poorly effective. The antifungal activity displayed by the examined plants suggests that they may have potential as a new natural fungicide for managing fungal pathogens and can be further utilized for the preparation of natural bio-pesticides. These phytoextracts have been emerged as safe alternatives to replace chemical fungicides and can be used as eco-friendly fungicides. Further work is required to increase the efficacy of these plant extracts in the field and also to determine the biologically active ingredient present in extracts as well as its mode of action.

Declarations

ACKNOWLEDGEMENT

We would like to extend our gratitude to Dr. Mohd Yaqub Bhat and Professor Abdul Hamid Wani of Mycology and Plant Pathology Laboratory, Department of Botany, University of Kashmir for their support in completing this task during the course of the study.

Ethical statement

The collection and handling of plant materials in this study complied with all the relevant institutional, national, international guidelines and legislation. This confirms adherence to necessary ethical and legal standards for plant research.

Author contributions

Mansoor Ahmad Malik: Conceptualization, Methodology, Data collection, Curation and

Analysis of Software, Visualization, Writing-Original draft. **Nusrat Ahmad**: Data collection, Curation and analysis, Writing-Original draft. **Mohd Yaqub Bhat**: Review and Editing, Conceptualization, **Abdual Hamid Wani**: Supervision, Investigation. All authors contributed to the article and approved the submitted version.

Funding

The authors received no financial assistance during the study period.

Data availability

The data used in this manuscript is available in the manuscript.

Competing interest

The authors declare they have no competing interests.

Ethical Responsibilities of Authors

All authors have read, understood, and have complied the submitted version.

Ethics approval and consent to participate

Not applicable.

Conflict of interest

None

References

1. Abdel Ghany T.M. (2014). Eco-friendly role of *Juniperus procera* as safe alternative for controlling fungal growth and their secondary metabolites. The African Journal of Mycology and Biotechnology, 19 (1), 21-36.
2. Abdel Ghany T.M. and Hakamy O.M. (2014). *Juniperus procera* as food safe additive, their antioxidant, anticancer and antimicrobial activity against some food-borne bacteria. J.Biol. Chem. Research., 31(2): 668-677.
3. Abdulghaffar O.; Abbas H.; Karim M. (2010). Samira Ebrahimzadeh Antifungal Effects of Thyme, Agastache and Satureja Essential Oils on *Aspergillus fumigatus, Aspergillus flavus* and *Fusarium solani* Veterinary Research Forum Vol: 1, No: 2, pp. 99 – 105.
4. Adandonon, A., Aveling, T.A.S., Labuschagne, N., Tamo M. (2006). Biocontrol agents in combination with Moringa oleifera extract for integrated control of *Sclerotium*-caused cowpea damping-off and stem rot. European Journal of Plant Pathology, 10, 1-6.
5. Alkhail, A.A. (2005). Antifungal activity of some extracts against some plant pathogenic fungi. Pakistan Journal of Biological Sciences, 8(3), 413-417.
6. Aly AA, Omar SA, Zayed SME, Mansour MTM, (2000). Use of saponin- containing A triplex nummularia to suppress damping of cotton seedling. J. Agric. Sci. Mansura Uni., 25:7621-7631.
7. Aly MM, Bafiel S, (2008). Screening for antimicrobial activity of some medicinal plants in Saudi Arabia. World conference on medical and aromatic
8. Bowers, J.H and J.C. Locke. (2000) Effect of botanical extracts on population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the green house. Plant Dis., 88:300-305.
9. Buch, H. and Arya A. (2015). Antifungal activity of selected plant extracts against three pathogenic fungi of *Gossypium herbaceu. Current Research in Environmental and Applied Mycology*, **7** (2): 103–

108.

10. Daferera, D.J, B.N. Zirgas and M.G. Polission. (2000). GCMS Analysis of essential oil from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. J. Agric. Food. Chem.,48: 2576-2581.
11. Dhingra, O.D., M. L.N. Costa, G.J. Silva and E.S.G. Mizubuti. (2004). Essential Oil of Mustard to control *Rhizoctonia solani* causing seedling damping off and seedling blight in nursery. Fitopatologia brasileira, 24: 683-686.
12. Fawzi, E. M., Khalil, A. A. and Afifi, A. F. (2009). Antifungal effect of some plant extracts on *Alternaria alternata* and *Fusarium oxysporum* African Journal of Biotechnology Vol. 8 (11), pp. 2590-2597.
13. Gwa, V.I., Nwankiti, A.O. and Ekefan, E.J. (2018). Antifungal effect of five aqueous plant extracts on mycelial growth of *Penicillium expansum* isolated from rotted yam tubers in storage. *Acta Scientific Agriculture*, **2**(6): 65-70.
14. Houghton, P.J., K.M. Ismail, L. Maxia and G. Appendino. (2006). Antidermatophytic prenglated coumarins from asafoetida. Planta Med., 72 DOI; 10.1055/S – 949741.
15. Imtiaj, A., Syed, A. R., Shahidul, A., Rehana, P., Khandaker, M. F., Sang-Beom, K., Tae-Soo, L. (2005). Effect of Fungicides and Plant Extracts on the Conidial Germination of *Colletotrichum gloeosporioides* Causing Mango Anthracnose, Mycobiology 33(4), 200-205.
16. Jobling, J. (2000). Essential oils: A new idea for postharvest disease control. Sydney Postharvest Laboratory information sheet. (http://www.postharvest.com.au/gfv_oils.pdf).
17. Kaur, R. A. J. B. I. R., & Arora, S. A. R. O. J. (2015). Alkaloids-important therapeutic secondary metabolites of plant origin. *J Crit Rev*, **2**(3): 1-8.
18. Kazmi, S.A.R., S. Shahzad and I. Niaz. (1995). Effect of neem oil on in vitro growth of root infecting fungi. Pak. J. Bot., 27(1): 217-220.
19. Khandare, K. R. and Vasait, R. D. (2017). Use of Biopotentials of plant extracts of medicinal importance against pathogenic fungi *Fusarium oxysporum*. *Bioscience discovery*, **8**(1): 87-92.
20. Koka, J. A., Wani, A. H., Bhat, M. Y. and Parveen, S. (2017). Antagonistic activity of *Trichoderma* spp. against some fungi causing fungal rot disease of brinjal. *Trends in Biosciences*, **10**(16): 2844-2846
21. Koka, J. A., Wani, A. H., Bhat, M. Y., & Parveen, S. (2017). Antifungal activity of ethanolic and aqueous leaf extracts of Taraxicum officinale and Mentha arvensis on the growth of some selected fungal species under in vitro conditions. *Int. J. Pure Appl. Biosci*, *5*, 1170-1176.
22. Koka, J.A., Wani, A.H., Bhat, M.Y., Parveen, S., Fazili, M.A. and Ahmad, N. (2019). Antifungal activity of selected plant extracts against *Trichotheciumroseum* (Pers.) Link (1809) (Sordariomycetes: Hypocreales), causal organism of fungal rot of *Solanum melongena* L. (Solanales: Solanaceae) in Kashmir, India. *Brazilian Journal of Biological Sciences*, **6**(12): 123-132.
23. Locke, J.E. (1995). Fungi. In: The Neem Tree, source of Unique National Products for Integrated pest Management, Medicine, Industry and Other proposes. (Ed.): H. Schmutterer. V.C.H, Weinheim, Germany. p 118-125.

24. Menghani, K. and Sharma, S. K. (2012). Antimicrobial activity of *Juniperus communis* and *Solanum xanthocarpum*. International Journal Pharmacetical Sciences and Research, 3(8), 2815-2818.
25. Momin R.A. and Nair M.G. (2001). Mosquitocidal Nematicidal and antifungal compounds from *Apium graveolens* L., seeds. J. Agric. Food Chem., 49: 142-145.
26. Negri, M., Salci, T. P., Shinobu-Mesquita, C. S., Capoci, I. R., Svidzinski, T. I., & Seki Kioshima, E. (2014). Early state research on antifungal natural products. *Molecules*, *19*(3), 2925-2956.
27. Obagwu, J., Korsten, L. (2003). Control of citrus green and blue molds with garlic extracts. European Journal of Plant Pathology, 109, 221–225.
28. Omar SA, Abd-El-Halim AZ, (1992). Fungal growth response to alfalfa (Medicago sativa L.) saponin extract. Egyptian J. Appl. Sci., 7: 24-32.
29. Paola, D.D, Cabrera, A., Alem, D., Larranaga, P., Ferreira, F. and Rizza, M.D. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. *Chilean Journal of Agricultural Research*, **71**(2).
30. Parveen, S., Wani, A. H., Bhat, M. Y. and Koka, J. A. (2016) Biological control of postharvest fungal rots of rosaceous fruits using microbial antagonists and plant extracts – a review. *Czech Mycology,* **68**(1): 41-66.
31. Parveen, S., Wani, A.H., Bhat, M.Y., Malik, A.R., Koka, J.A. and Ashraf, N. (2017). Antimycotic potential of some phytoextracts on some pathogenic fungi. *Journal of Biopesticides*, **10**(1): 60-65.
32. Rahber-Bhatti, M H., (1988). Antifungal property of plant leaf decoctions for leaf rust of wheat Pak. J. Bot., 20(2): 259-263.
33. Raji, R. and Raveendran, K. (2013). Antifungal activity of selected plant extracts against phytopathogenic fungi *Aspergillus niger*. *Asian Journal of Plant Sciences and Research*, **3**:13-15.
34. Ramaiah, A. K., & Garampalli, R. K. H. (2015). In vitro antifungal activity of some plant extracts against Fusarium oxysporum f. sp. lycopersici. *Asian Journal of Plant Science and Research*, *5*(1), 22-27.
35. Saadabi AMA, (2006). Antifungal activity of some Saudi plants used in traditional medicine. Asian J. Plant Sci., 5: 907-909.
36. Santas, J., Almajano, M. P. and Carbo, R. (2010). Antimicrobial and antioxidant activity of crude onion (*Allium cepa* L.) extracts. *International Journal of Food Science Technology,* **45**: 403–409.
37. Satish, N., Sultana, S., and Nanjundiah, V. (2007). Diversity of soil fungi in a tropical deciduous forest in Mudumalai, Southern India. *Current Science*, 669-677.
38. Sesan, T.E., Enache, E., Iacomi, B.M., Oprea, M., Oancea, F. and Iacomi, C. (2015). Antifungal activity of some plant extracts against *Botrytis cinerea* Pers. in the blackcurrant crop (*Ribes nigrum* L.). *Acta ScientiarumPolonorumHortorum Cultus*, **14**(1): 29-43.
39. Shelef LA, (1983). Antimicrobial effects of spices. J. Food Safety, 6: 29- 44.
40. Sridhar, S.R; R.V. Rajagopal, R. Rajavel, S. Masilamani and S.Narasimhan. (2003). Antifungal activity of some essential oils. J. Agric. Food. Chem., 51: 7596-7599.

41. Taskeen-Un-Nisa, Wani, A.H and Mir, R.A. (2010). Antimycotic activity of plant extracts on the spore germination of some pathogenic fungi. *Mycopathology*, **8**: 65-69.
42. Thyagaraja, N and A. Hosono. (1996). Effect of spice extract on fungal inhibition. Lebensmittel Wissenchaftund–Technologie., 29: 286-288
43. Tumen, I., Fred, J.E., Carol, A.C., Jeffery, A.T. (2013). Antifungal activity of heartwood extracts from three Juniperus species. BioResource, 8 (1)12-20.
44. Zaker, M. and H. Mosallanejad, (2010). Antifungal activity of some plant extracts on *alternaria alternata,* the causal agent of *alternaria* leaf spot of potato. Pak. J. Biol. Sci., 13: 1023-1029.
45. Znini, M., Cristofari, G., Majidi, L., Mazouz, H. A. M. I. D., Tomi, P., Paolini, J. U. L. I. E. N., and Costa, J. (2011). Antifungal activity of essential oil from Asteriscus graveolens against postharvest phytopathogenic fungi in apples. *Natural product communications*, **6**(11): 1934578X1100601147.

Tables

Tables 1 to 10 are available in the Supplementary Files section

Figures

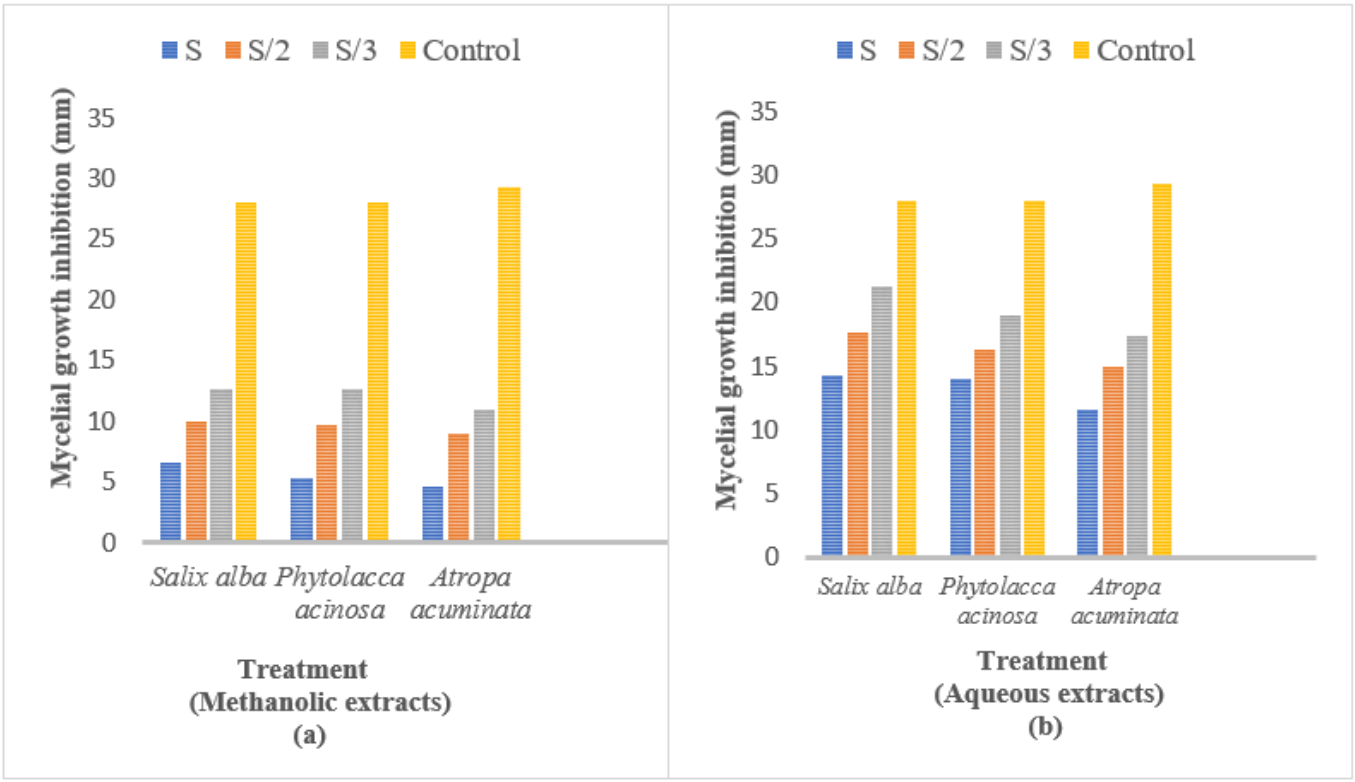

Figure 1

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Alternaria alternata*

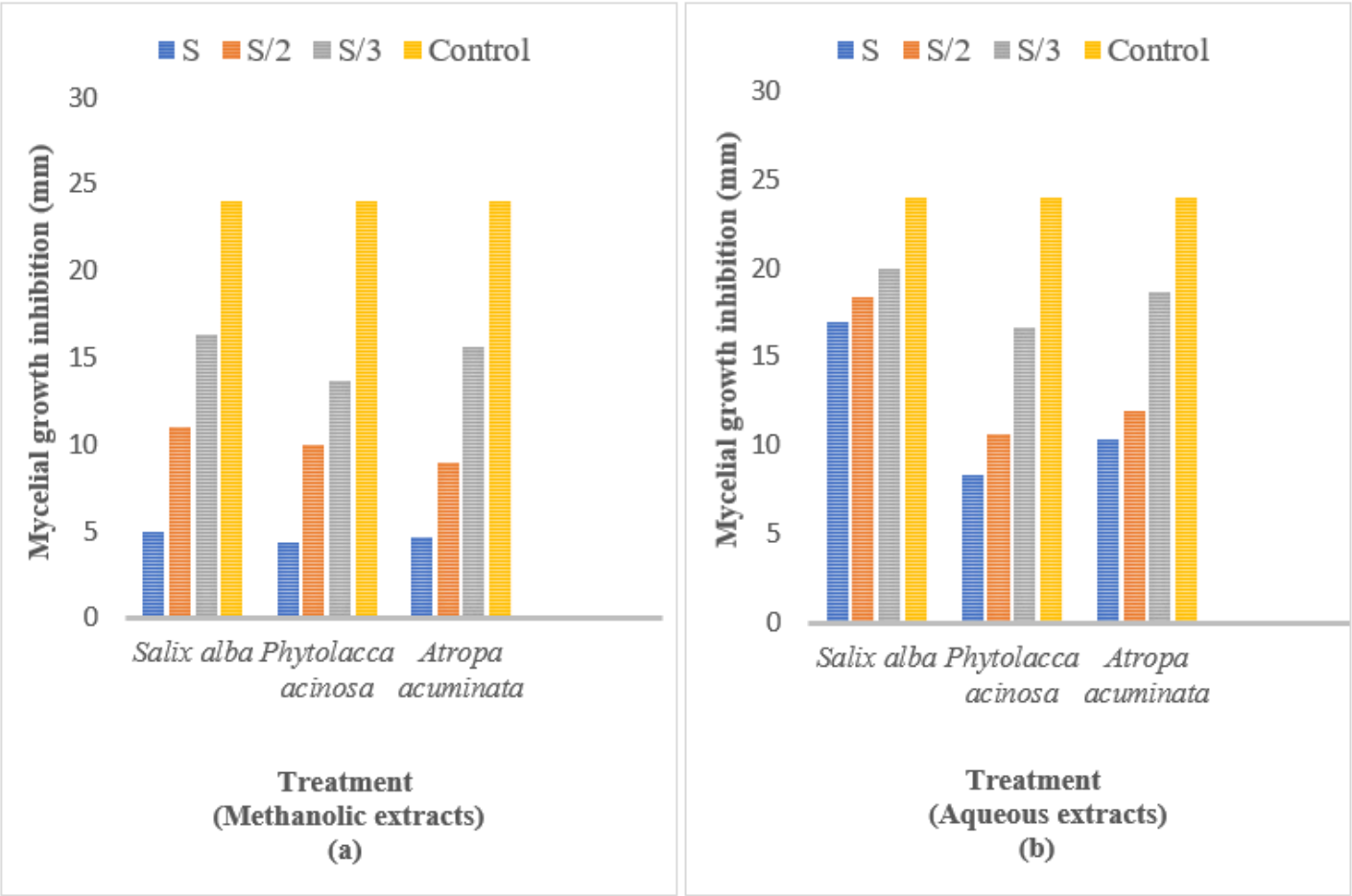

Figure 2

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Fusarium oxysporum*

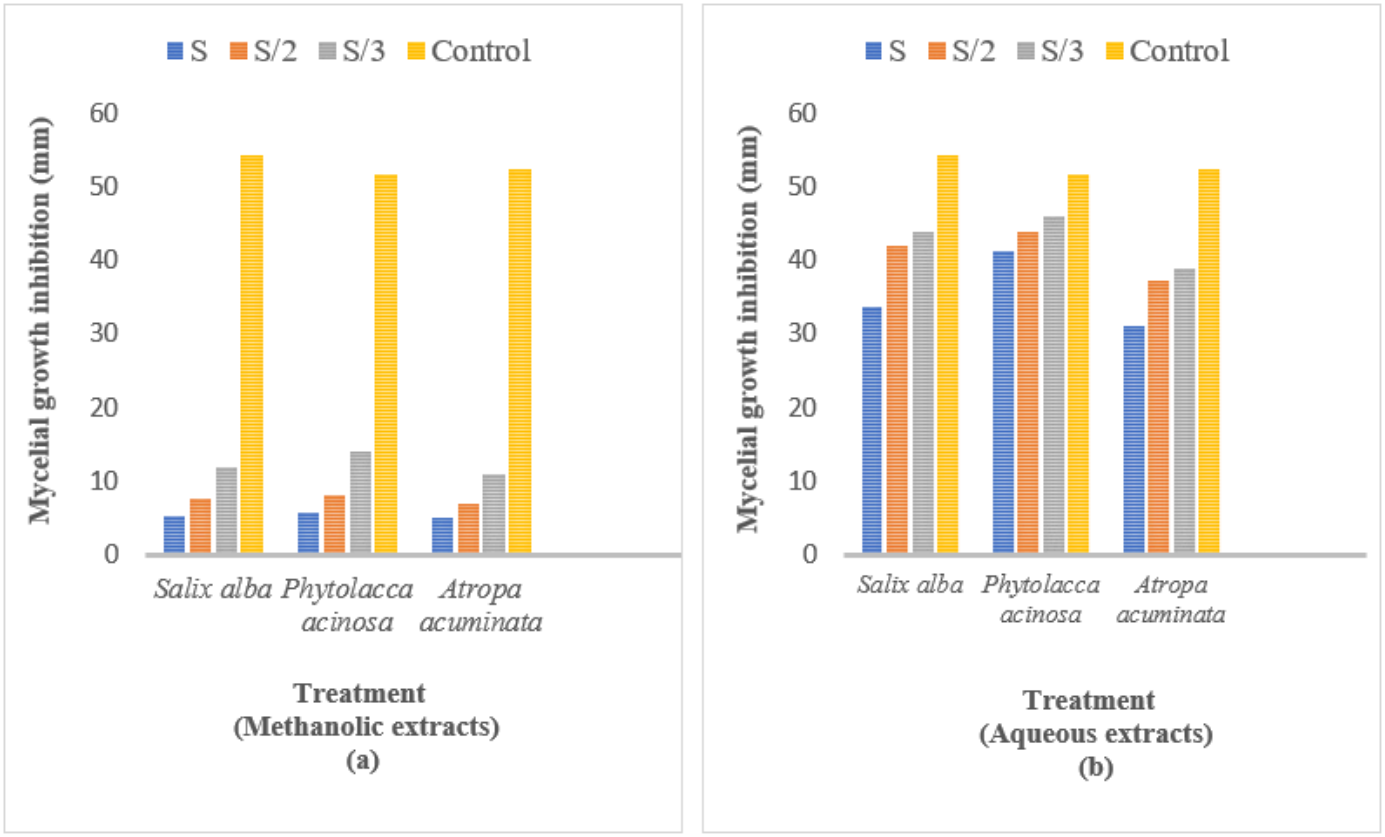

Figure 3

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Rhizoctonia solani*

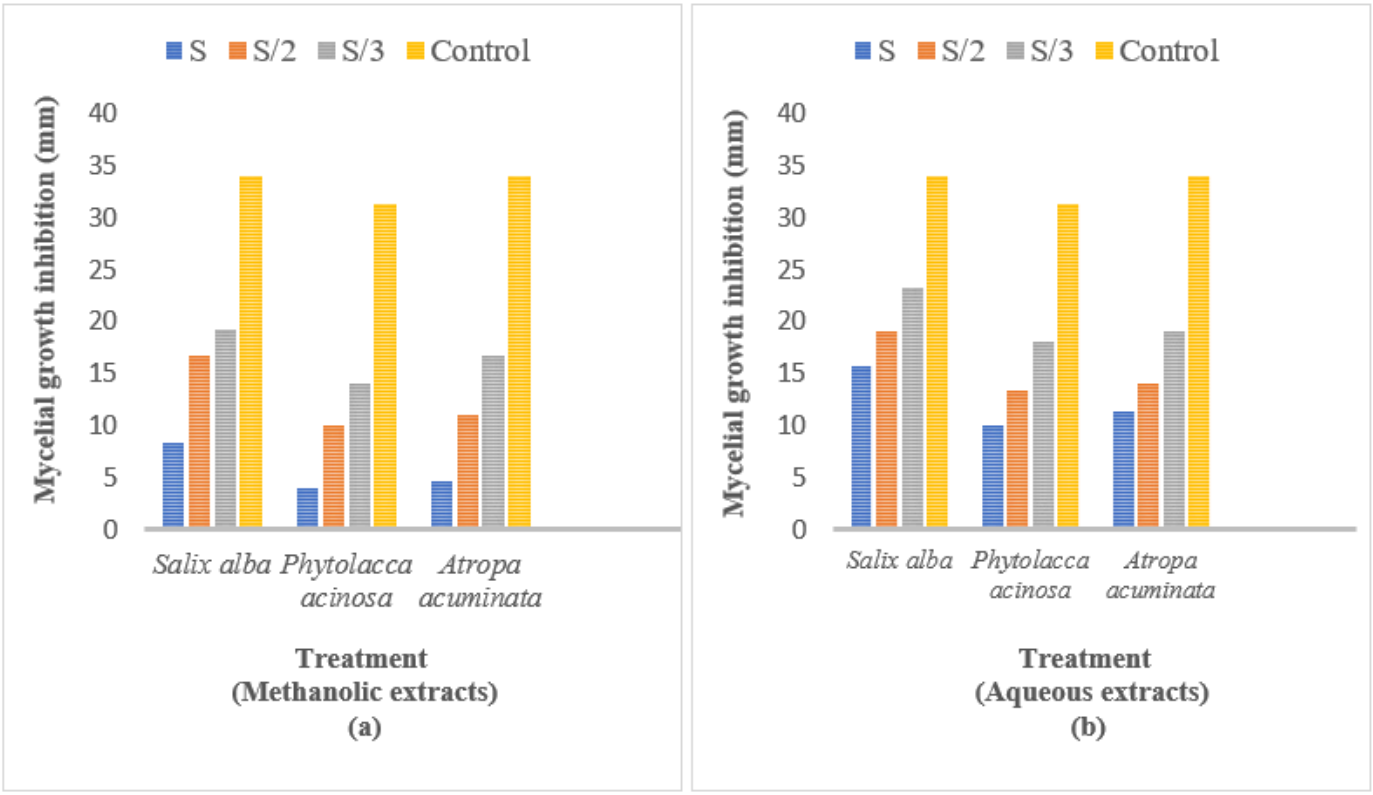

Figure 4

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Cladosporium cladosporioides*

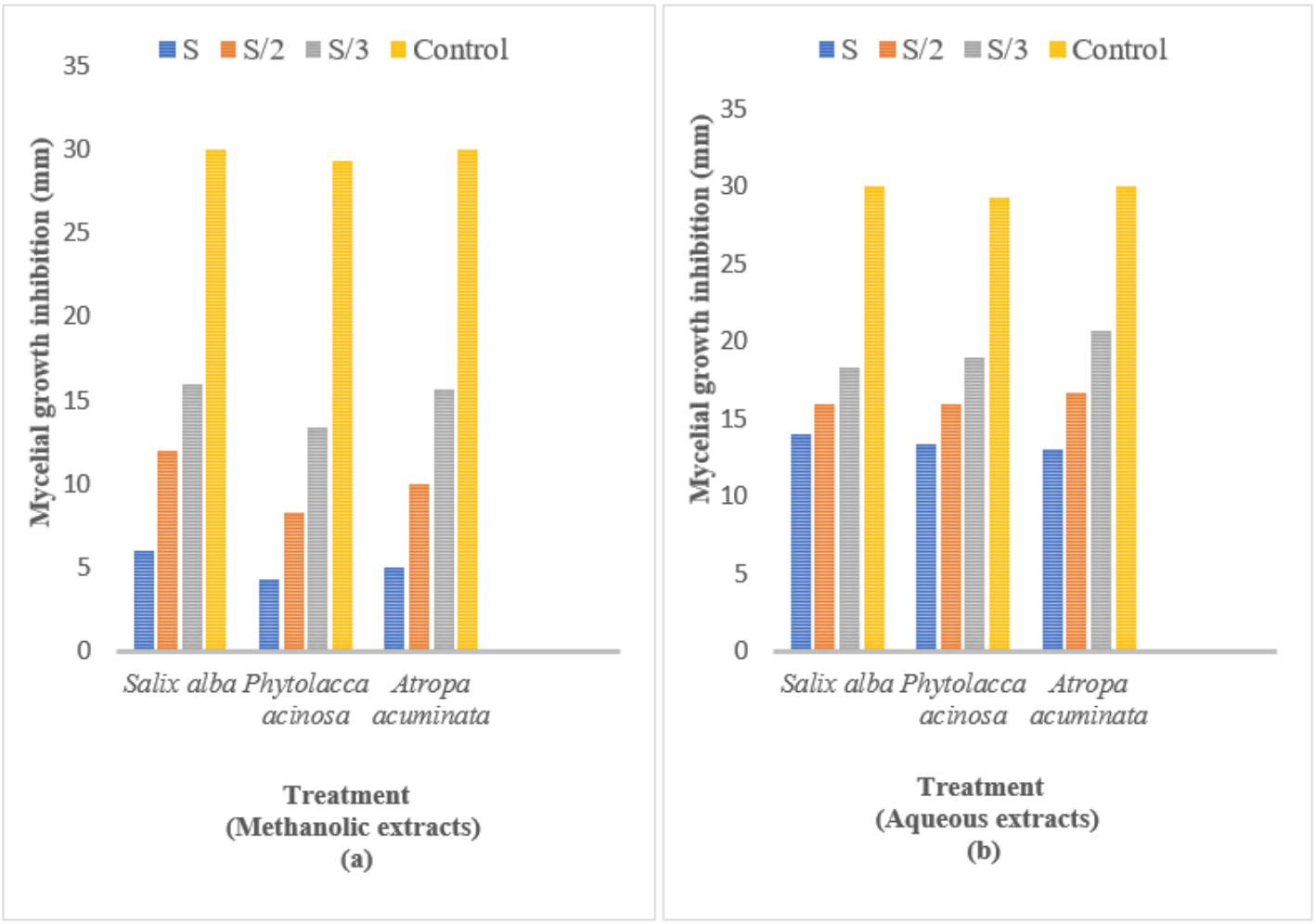

Figure 5

Efficacy of various concentrations of some phytoextracts on mycelial growth inhibition of *Penicillium citrinum*

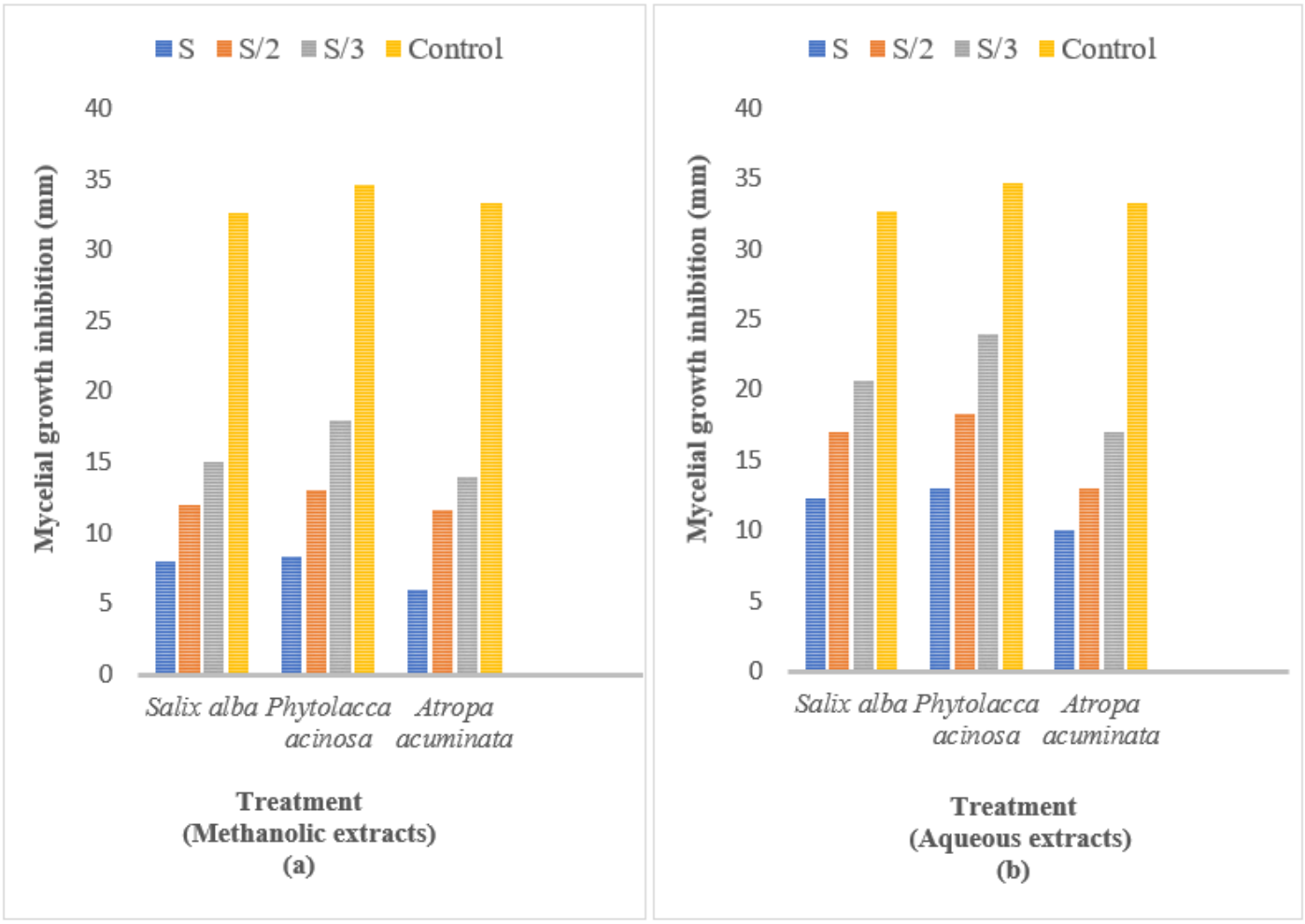

Figure 6

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Curvularia lunata*

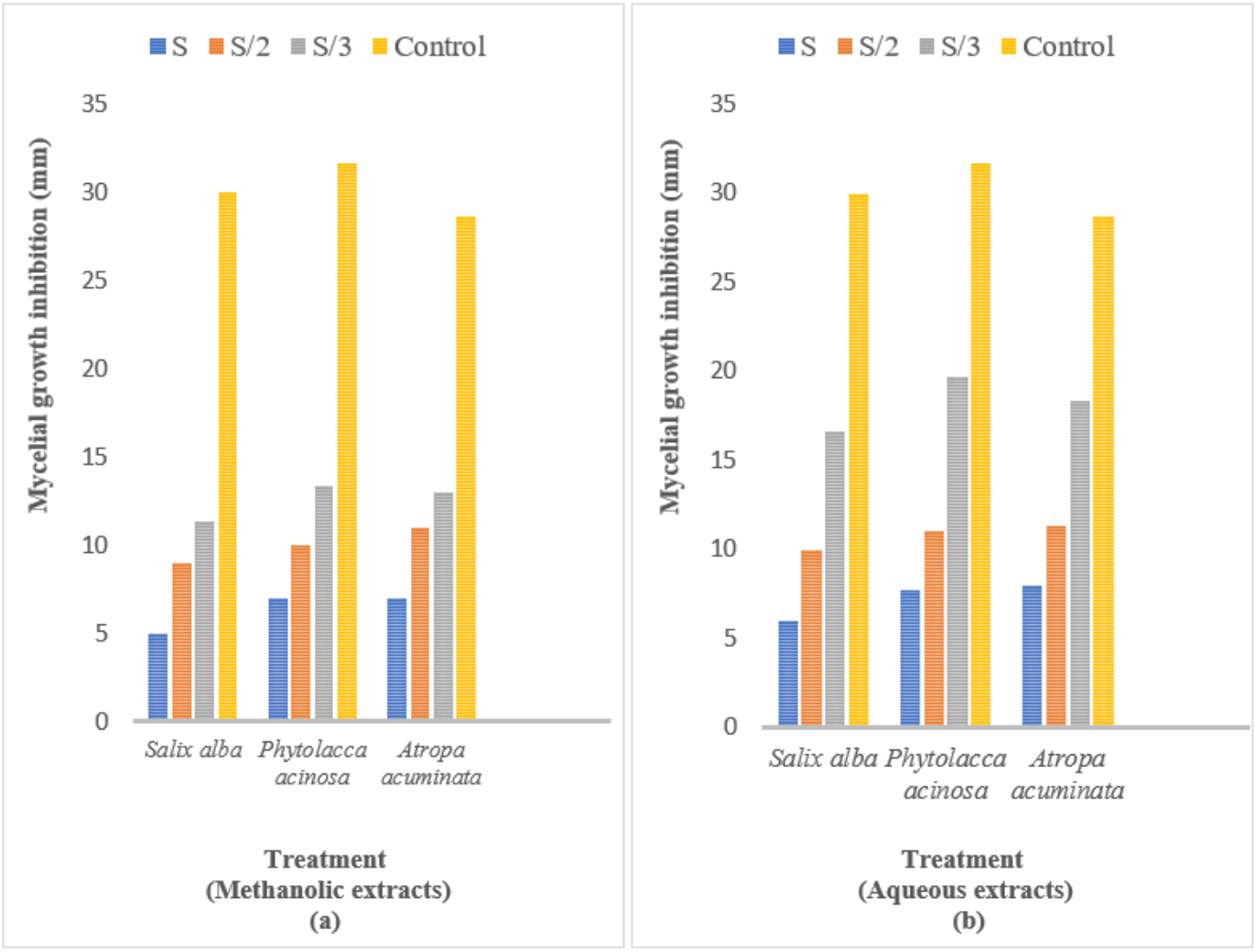

Figure 7

Efficacy of various concentrations of some phytoextracts on the mycelial growth inhibition of *Fusarium metavorans*

Supplementary Files

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