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Research Article

Eco-Friendly Potential of selected medicinal plants extracts for Suppressing *Fusarium oxysporum* Causal Agent of Fusarium Wilt Disease in *Solanum lycopersicum*

***Mahammad Omer, Gulab Khan and Abdullah Hussaini**

Article Info

Department of Horticulture,
Ghazni University,
Gardiz-Ghazni Street ,Qala Jawz Ghazni, Afghanistan
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*Email:
omermohdafghan@gmail.com

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Abstract

In this research, we have used the medicinal plant extracts as a antifungal agents of *Fusarium oxysporum* Causal Agent of Fusarium Wilt Disease in *Solanum lycopersicum*, which is common problem in the Tomato cultivation in Afghanistan. Comparative Antifungal activity of *Shilajit*, *Datura metel* L. and *Allium sativum* L. Fresh and healthy leaves, stems, of *Shilajit*, *Datura metel* L. and *Allium sativum* L. were collected from Nisan area of Herat province of Afghanistan, in December 2023. To handle Fusarium wilt disease in an eco-friendly and sustainable way, we examined the antifungal activity of Chloroform (CHCl), ethyl acetate (EtOAc), and butanol extracts and dilutions of chosen medicinal plants available in our region. The current study found that *Datura metel* L. Extract was more efficient against FORL than the other medicinal extracts (*Shilajit* and *Allium sativum* L. Extract). Similarly, ethyl acetate extract was shown to be substantially more effective in inhibiting *Fusarium oxysporum* growth than chloroform and butanol extracts, which had comparable effects. Furthermore, at the levels used, it was shown that the greatest concentration hindered mycelial growth of the targeted pathogen the most. Present result showed that ethyl acetate extract of *Datura metel* L. were more effective

Keywords: *Shilajit*, *Datura metel* L., *Allium sativum* L., Chloroform (CHCl), ethyl acetate (EtOAc), butanol (n-BuOH), antifungal activity**.**

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Introduction

The agricultural industry, despite undergoing revolutionary transformations, continues to face challenges posed by climate change and the increasing demands for food security [1, 2]. Afghanistan is a landlocked country in the heart of South and Central Asia and the wider Middle East, between latitudes 29.5N-38.5N and longitudes 60.5E-75E. Many causes have contributed to the destruction of Afghanistan's agricultural economy in the past several decades. First and foremost, multiple years of conflict and political instability have decimated the majority of these industries, along with their infrastructure. High temperatures are one of the most important problems throughout the country, particularly in the eastern regions. Agro-chemicals, including pesticides, fungicides, and bactericides, are used extensively in the nation. Crop protection depends heavily on chemical-based insecticides. In this research we have use the medicinal plant extracts as a antifungal agents of *Fusarium oxysporum* Causal Agent of Fusarium Wilt Disease in *Solanum lycopersicum*, which is common problem in the Tomato cultivation.

Tomato (*Solanum lycopersicum* L.) family Solanaceae is considered as a significant crop worldwide among the vegetables. Tomatoes contain important minerals, vitamins, sugars & the most important anti-oxidant lycopene [3]. Due to the attack of several viral & soil borne microbial pathogens, the yield per hectare of tomato is badly affected. In nurseries where young vulnerable transplants are produced damping-off caused by R. solani, causes substantial losses [4]. Use of synthetic fungicides is mainly practiced for management of wilt disease [5]. This measure may cause adverse effects on the environment and human health [6]. Furthermore, the presence of pesticide residue in the fruit may reduce product quality. The increasing recognition of the environmental risks connected with fungicides has prompted the quest for non-conventional biologically derived compounds for disease treatment. As a result, it is a time-consuming endeavor to find an alternate method of controlling plant diseases utilizing natural plant components. Controlling fungal infections in crops involves adopting resistant cultivars, extended rotations, and fumigants. Plant-derived chemicals are being explored as alternatives to synthetic fungicides for controlling fungal infections. Agapanthus africans leaf extracts exhibit antifungal properties [7]. Plant compounds are poisonous to a wide range of fungal and bacterial diseases. Recently, over 500 plant species were tested for antifungal activities [8]. Only 3% of plants studied had significant antifungal efficacy. Many writers have explored the role of secondary metabolites in fungal inhibition. The antifungal action of *Quillaja saponaria* extract may be attributed to the presence of saponins and phenolic chemicals [9]. Recent efforts have focused on developing environmentally safe, long lasting and effective measures such as the use of plant metabolites and plant-based treatments as promising alternatives to some chemical fungicides in particular hymexazol, benomyl and captafol which are currently used for Fusarium Crown and Root Rot control [10.11]. Some plants produce a variety of secondary metabolites with antifungal properties against some phytopathogenic fungi [12-14]. Wellknown examples of these bioactive compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins [15-17]. In this regard, plant-derived natural products have been exploited as active ingredients of biofungicides [18]. Some botanical extracts from *Azadirachta indica A. Juss., Calotropis procera* (Ait) R. Br., *Citrullus cololcynthis* (L.) *Schrad., Datura stramonium* (L.), and *Nicotiana tabacum* (L.) were effective in reducing *Fusarium oxysporum* mycelial growth [19]. *Cinnamomum burmanni* (Ness ex Blume) leaf aqueous extract efficiently suppressed radial growth, biomass formation, and spore formation of *F. oxysporum f.* sp. *lycopersici*

[20]. Fusarium Crown and Root Rot (FCRR) disease caused by *Fusarium oxysporum* is one of the most damaging soilborne diseases occurring worldwide and representing a serious threat both in protected and open field tomato crops [21]. FCRR accounts for approximately 70–83% loss of tomato plants, attributed to root rot and basal stem decay, as well as the eventual death of severely infected plants [22]. Yield reductions have been estimated at up to 15–95% [23,24].

Although *Fusarium oxysporum* is commonly characterized as a soil-borne pathogen in tomato plants, [25] determined that this fungus may infect tomato crops through infected seeds. Fungal colonization of the host plant's xylem causes obstruction and disintegration, resulting in wilt disease symptoms such as leaf withering, yellowing, and, finally, plant mortality [26,27]. Management of *Fusarium oxysporum* is essential since this disease and its various specific forms harm a wide range of economically valuable crops. Management of phytopathogenic diseases by nontoxic anti-fungal agents like extracts of plants is an environment friendly alternative over harmful pesticide [28,29]. The use of plant materials as natural constituents could be a substitute to fungicides [30]. Plant extracts are usually comprise of many secondary metabolites like terpenoids, saponins, alkaloids phenolic, & flavonoids glycosides possess strong antifungal effects [31]. Several higher plants & their constituents have been reported to control a number of pathogens that cause severe plant diseases [32-36].

Shilajit, Datura metel L. and *Allium sativum* L.

Shilajit also known in the north of India as *salajit*, *shilajatu*, *mimie*, or *mummiyo* is a blackish-brown powder or an exudate from high mountain rocks, especially in the Himalayans mountains between India and Nepal, although it has been also found in Russia, Tibet, Afghanistan, and now in the north of Chile, named as *Andean Shilajit* [37]. Considering its unique composition as a phytocomplex, very rich in fulvic acid, researchers hypothesize that *Shilajit* is produced by the decomposition of plant material from species such as *Euphorbia royleana* and *Trifolium repens* [38,39]. *Shilajit* is composed mainly of humic substances, including fulvic acid, that account for around 60% to 80% of the total nutraceutical compound plus some oligoelements including selenium of antiaging properties [40,41]

Shilajit is composed of three primary chemical units namely, (1) low and medium molecular weight non-humic organic compounds comprising free and conjugated (e.g. fattyacyl, aminoacyl, lipoidal), dibenzo-α-pyrones. (2) Medium and high molecular weight DCPs (dibenzo-α-pyrones-chromoproteins),

containing trace metal ions and colouring matter such as carotenoids and indigoids and (3) metallo-humates like fulvic acids and fusims with dibenzo-α-pyrones in their core nuclei [42]. The chemical content of *Shilajit* is controlled by several factors such as adjacent plant-species, geological environment of the rock and soil, temperature, humidity and altitude, etc. [42]. For example, *Shilajit* obtained from India in the region of Kumoan contains a higher percentage of fulvic acids (21.4%) compared with *Shilajit* obtained from Nepal (15.4%), Pakistan (15.5%) and Russia (19.0%). However, the bioactive low molecular compound was found in high quantities in *Shilajit* obtained from Nepal. Similarly, humic constituents in *Shilajit* samples obtained from these countries also varied [43]. *Datura metel* the member of Solanaceae family is famous due to its herbicidal, anti- bacterial & anti-fungal activity [44]. This plant contains tropane, withanolide, trigloyl esters of tropine, calystegines & pseudotropine alkaloids [45,46].

Materials and methods

Isolation and Identification of the Pathogen

The infected parts of tomato plant were collected in the polythene bags, which were made airtight. Collected materials were labeled properly and then brought to the Laboratory of Department of Horticulture, Ghazni University,

Gardiz-Ghazni Street ,Qala Jawz Ghazni, Afghanistan. The pathogen was isolated on potato dextrose agar (PDA) medium [47]. The infected parts of tomato plant were cut into small pieces. The pieces were then washed in running tap water, sterilized in 0.1 percent mercuric chloride solution and washed repeatedly for several times in sterilized distilled water to remove mercuric chloride solution. Three pieces were transferred to PDA plate. Plates were incubated at 25˚C ± 2˚C for 15 days for recovery of pathogen. *Fusarium oxysporum* was purified by single spore method and according to morphological characteristics; identification was done with the help of standard keys [48,49].

Maceration: This process is conducted by soaking the plant materials (coarse or powered) in a closed stoppered container in a solvent allowed to stand at room temperature for 2–3 days with frequent stirring to obtain plant extracts. A sealed extractor is used to avoid solvent evaporation at atmospheric pressure. The process is intended to soften and break the plant's cell walls to release the soluble phytoconstituents. The mixture is then pressed or strained by filtration or decantation after a specific time [50,51]. Maceration is the simplest and still widely used procedure. The extraction procedure in this stationary process works on principle of molecular diffusion, which is a time-consuming process. Maceration ensures dispersal of the concentrated solution accumulation around the particles' surface and brings fresh solvent to the surface of particles for further extraction [52].

Collection and processing of *Shilajit, Datura metel* L. and *Allium sativum* L.

Fresh and healthy leaves, stems, of *Shilajit*, *Datura metel* L. and *Allium sativum* L. were collected from Nisan area of Herat province of Afghanistan, in December 2023. Fresh materials were washed thoroughly under running tap water to remove any dust, and were dried at 30°C for 72 h and grounded into fine powder before being used for extraction. Each plant material was size reduced using the blender. After that sieved in separately all the plant materials it was using sieve no 60. Each blended and dried powder of individual plant materials was weighed in the required quantity. Individual plant materials were macerated in a hydroalcoholic solvent for 72 hours. After 72 hours, the separate suspensions were passed through a fine muslin fabric cloth, and the collected filtrate was evaporated to dryness and stored in a desiccator. The yield of each plant material gathered formulation was determined and stored separately in an airtight container for further research. [53] 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.

Determination of extraction yield

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

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

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

In-Vitro Assessment:

Antifungal activity assay of plants extracts by using poison food technique

Shilajit, Datura metel L. and *Allium sativum* L. organic extracts were assessed for their ability to suppress in vitro growth of *Fusarium oxysporum*. Plant extracts, which could suppress the fungal growth, were tested for their efficiency against the pathogen by using an agar dilution technique. Five ml of each extract concentration was added with 95 ml of molten Potato Dextrose Agar (PDA). Thus obtained concentration of 5%, 15%, 25%, and 35% extract to a PDA medium. After the

solidification of the medium, 1 cm diameter of mycelia block from 7-day-old colony of *F. oxysporum* was inoculated in the center of each Petri plate. Radial growth was measured each day, starting 2nd days after incubation in the dark at 25°C, until the 5th day. The percentage growth inhibition of each extract was calculated by the formula:

% inhibition = [growth in control - growth in sample/ growth in control] × 100.

Three replicates in a completely randomized design were used within each treatment. The media amended with Chloroform (CHCl), ethyl acetate (EtOAc), and butanol was considered as negative and while carbendazim-based fungicide (It is a *broad-spectrum systemic* fungicide with protective and curative action.) was used as positive control, respectively [54]

Statistical analysis:

Statistical analysis of results was performed using IBM SPSS version 20 and the MS Excel 2010. One way ANOVA (Analysis of Variance) at value $p \leq 0.001$ followed by Tukey's Post Hoc test with $p \leq 0.05$ was used to determine the significant differences between the results obtained in each experiment.

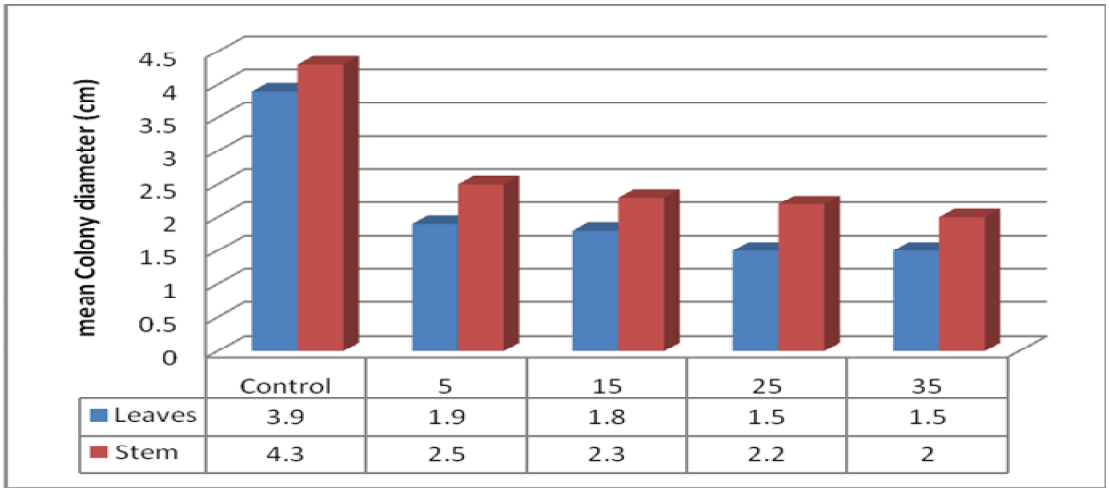
Results

Comparative Antifungal activity of *Shilajit, Datura metel* L. and *Allium sativum* L. To handle Fusarium wilt disease in an eco-friendly and sustainable way, we examined the antifungal activity of Chloroform (CHCl), ethyl acetate (EtOAc), and butanol extracts and dilutions of above medicinal plants available in our region. It was found that all *Shilajit, Datura metel* L. and *Allium sativum* L. extracts significantly reduced FCRR severity when compared to pathogen-inoculated. Maximum inhibition was found at 35% concentration of Ethyl Acetate extract of *Datura metel* L. against studied fungi.

Antifungal activity of *Shilajit, Datura metel* L. and *Allium sativum* L. Extract

Antifungal activity of *Shilajit* ectract:

The effect of *Shilajit* Extract were analysed with the concentration of 5,15,25,35% on *Fusarium oxysporum*, we observed that the 96.8% inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 96.5, using 15% the inhibition was 96.8, at Conc. of 25% inhibition was 97.5 and At 35% conc inhibition was 97.9 observe and recorded on 5th day of incubation.

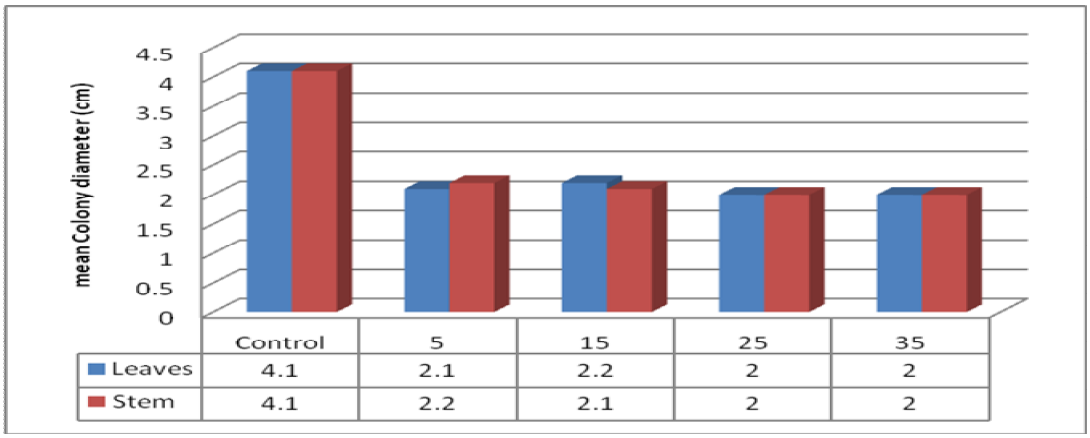


Extracts of *Shilajit* tested at different concentrations (%v/v)

Figure 1-A : Mean colony diameter (cm) of *Fusarium oxysporum* grown on PDA medium supplemented with different concentrations of aqueous extracts from *Shilajit* leaves and stems noted after 5th days of incubation at 25°C.

Antifungal activity of *Datura metel* L. Extract

The effect of *Datura metel* L. Extract were analysed with the concentration of 5, 15, 25, 35% on *Fusarium oxysporum*, we observed that the 98% inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 97.8, using 15% the inhibition was 97.8, at Conc. of 25% inhibition was 98 and At 35% conc inhibition was 98% observe and recorded on 5th day of incubation.

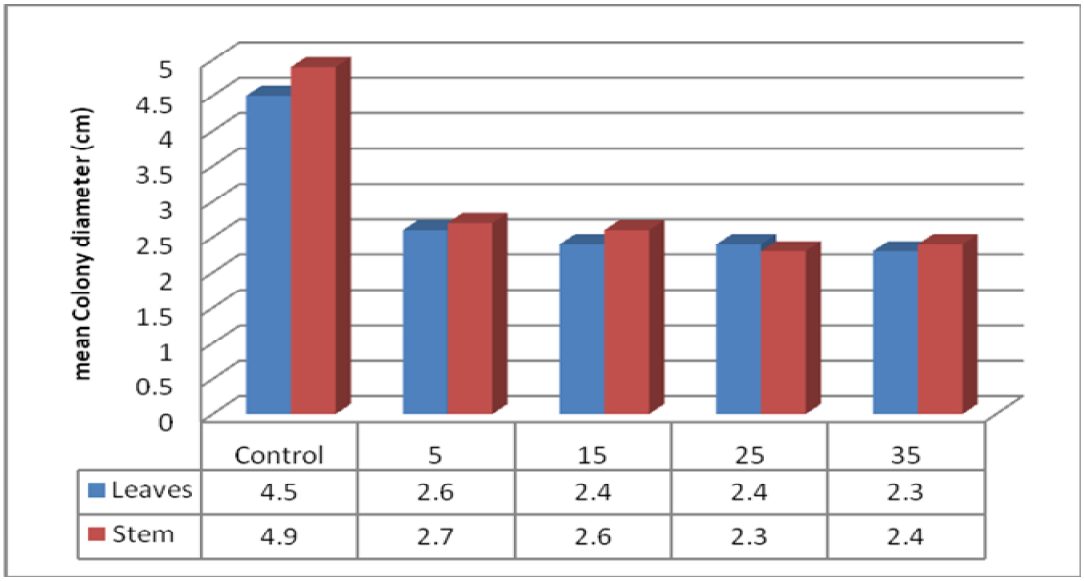


Extracts of *Datura metel* L. tested at different concentrations (%v/v)

Figure 1-B : Mean colony diameter (cm) of *Fusarium oxysporum* grown on PDA medium supplemented with different concentrations of aqueous extracts from *Datura metel* L. leaves and stems noted after 5th days of incubation at 25°C.

Antifungal activity of *Allium sativum* L. Extract

The effect of *Allium sativum* L. Extract were analysed with the concentration of 5,15,25,35% on *Fusarium oxysporum*, we observed that the 97.6% inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 97.3, using 15% the inhibition was 97.3, at Conc. of 25% inhibition was 97.7and At 35% conc inhibition was 97.7% observe and recorded on 5th day of incubation.



Extracts of *Allium sativum* L. tested at different concentrations (%v/v)

Figure 1-C : Mean colony diameter (cm) of *Fusarium oxysporum* grown on PDA medium supplemented with different concentrations of aqueous extracts from *Allium sativum* L. leaves and stems noted after 5th days of incubation at 25°C.

Figure 1(A,B,C) shows that all organic extracts tested demonstrated antifungal activity and suppressed pathogen radial development when compared to the control. The Extract of leaves shows more inhibition activity than the stem extract against FORL. It was shown that highest mycelial growth inhibition of the targeted pathogen was mainly reached at the highest concentration applied at 35%. In this regard, Uddin et al. [55] 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, all of which are known to have antimicrobial properties.

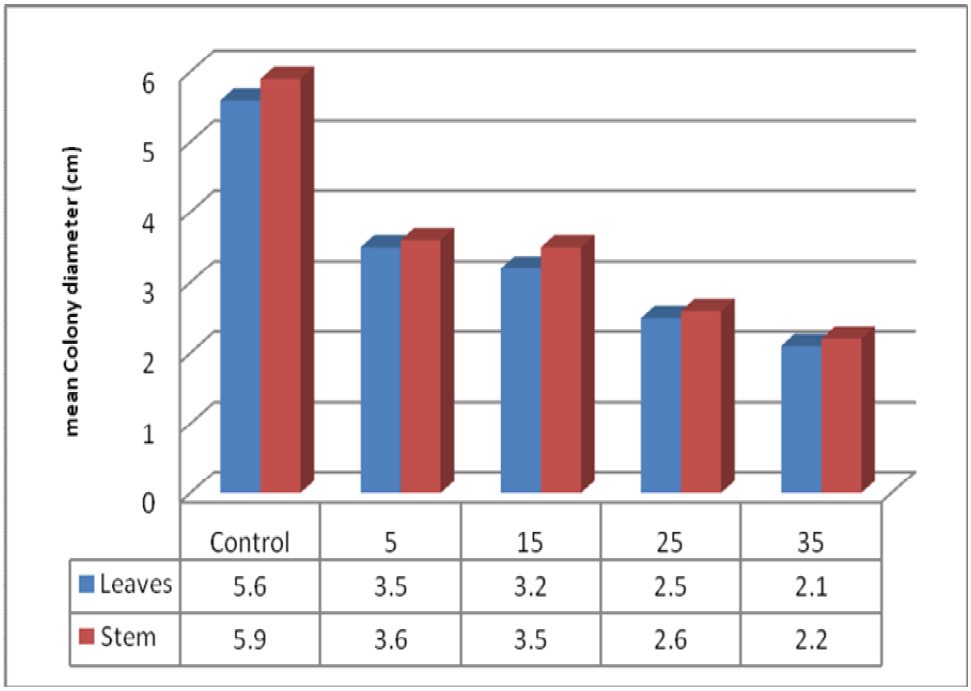
Antifungal activity of organic extracts of *Shilajit, Datura metel* L. and *Allium sativum* L.

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 oncentrations used. A significant interaction (at $P \leq 0.01$) between the three fixed factors was also detected.

To find out the inhibition of selected medicinal plant extracts against the *Fusarium oxysporum, we obtained the* organic solvents of namely Chloroform (CHCl), ethyl acetate (EtOAc), and butanol (n-BuOH) of each plant (solvent extracts of leaves and stem of plants).

Chloroform Extract

The effect of Chloroform Extract were analysed with the concentration of 5,15,25,35%on *Fusarium oxysporum*, we observed that the 96.8% inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 96.5, using 15% the inhibition was 96.8, at Conc. of 25% inhibition was 97.5 and At 35% conc inhibition was 97.9 observed and recorded on 5th day of incubation.

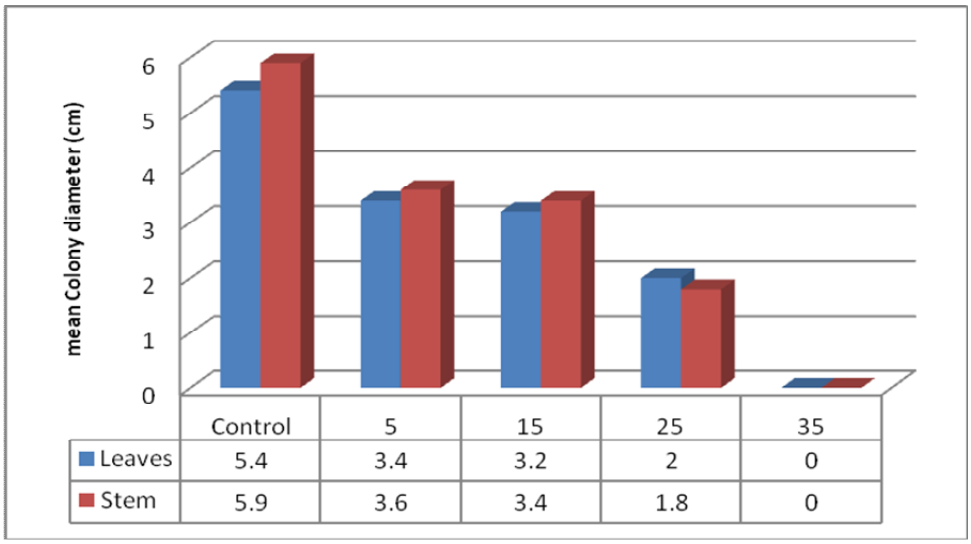


Chloroform Extract tested at different concentrations (%v/v)

Figure 2: Mean colony diameter of *Fusarium oxysporum* colonies grown on PDA medium supplemented with different concentrations of Chloroform Extract noted after 5th days of incubation at 25°C.

Ethyl Acetate Extract

The effect of ethyl acetate extract were analysed with the concentration of 5,15,25,35%on *Fusarium oxysporum*, we observed that the 100% inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 96.6, using 15% the inhibition was 96.8, at Conc. of 25% inhibition was 98.2 and At 35% conc. inhibition was 100% observed and recorded on 5th day of incubation.

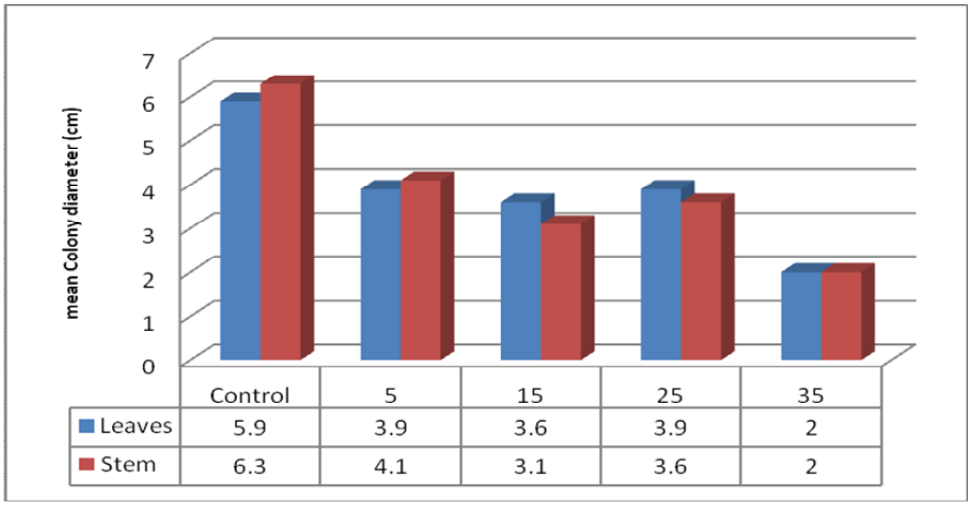


Ethyl Acetate extracts tested at different concentrations (%v/v)

Figure 3: Mean colony diameter of *Fusarium oxysporum* colonies grown on PDA medium supplemented with different concentrations of Ethyl Acetate extracts noted after 5 days of incubation at 25°C.

Butanol Extract

The effect of Butanol extract were analysed with the concentration of 5,15,25,35%on *Fusarium oxysporum*, we observed that the maximum inhibition of mean colony diameter in 35 % concentration. The inhibition of *Fusarium oxysporum* by using the 5% conc. were 96.1, using 15% the inhibition was 96.9, at Conc. of 25% inhibition was 96.1 and At 35% conc inhibition was 98% observed and recorded on 5th day of incubation.



Butanol extracts tested at different concentrations (%v/v)

Figure 4: Mean colony diameter of *Fusarium oxysporum* colonies grown on PDA medium supplemented with different concentrations of Butanol extracts noted after 5 days of incubation at 25°C.

The management of Fusarium wilt disease of tomato is mainly based on the application of chemical fungicides, crop rotation and the use of pathogen-resistant varieties. Application of chemical fungicides was a conventional method to control diseases caused by fungal pathogens, but tremendous health hazards have been reported from time to time during the application of chemical fungicides, in the field conditions [57]. However, fungicide application has resulted in the accumulation of residual toxicity in soil and vegetables, increase environmental pollution and alter the biological balance in the soil by decimating non - target and beneficial microorganisms [56].

The current study found that *Datura metel* L. Extract was more efficient against FORL than the other medicinal extracts (*Shilajit* and *Allium sativum* L. Extract). Similarly, ethyl acetate extract was shown to be substantially more effective in inhibiting *Fusarium oxysporum* growth than chloroform and butanol extracts, which had comparable effects. Furthermore, at the levels used, it was shown that the greatest concentration hindered mycelial growth of the targeted pathogen the most. Present result showed that ethyl acetate extract of *Datura metel* L. were more effective than the observation of Ambikapathy *et al.* [58] and Siva *et al.* [59].

Discussion

Finding novel fungicides that are effective, biodegradable, and selective is essential in order to address chemical-related issues. Natural plant-derived products are harmless and might be used into pest management programs since they have antifungal properties without being phytotoxic [60].

The use of solvents with variable polarities in the extraction operation is critical for successfully isolating substances with diverse polarities. It was also demonstrated that ethyl acetate extracts inhibited mycelial development the most at 35% concentration (100%) when compared to chloroform (98.2%) and Butanol fractions. All *Datura metel* L. Extract extracts inhibited *Fusarium oxysporum* mycelial growth at all doses tested. Both leaves and stem extract were shown to be the most active at 25%, resulting in 98% decreased growth compared to the untreated control. The extract of *Shilajit* leaves shows high inhibition activity against the *Fusarium oxysporum* than the stem extract. In *Datura metel* L. Extract of both the leaves and stem having equivalent inhibition against the the *Fusarium oxysporum.* The effect of *Allium sativum* L. leaves Extract were analysed with high inhibition against the *Fusarium oxysporum.* All three plant extracts effectively inhibited the pathogen's mycelial growth in culture. The results revealed that control was also the efficient at inhibiting the pathogen's mycelial radial growth. The current study found that plant extracts of *Shilajit, Datura metel L., and Allium sativum L.* were the most efficient at inhibiting the growth of the tested pathogen. The current study is the first to describe the antifungal activity of chloroform, ethyl acetate, and butanol extracts from *Shilajit, Datura metel L., and Allium sativum L.* Leaves and stems against *Fusarium oxysporum.* The results demonstrated that *Shilajit, Datura metel L., and Allium sativum L.* extracts had a robust antifungal impact on this disease. The most bioactive ethyl acetate extracts were discovered to be the leaves and stems, particularly when applied at the highest dose (35%). Plant extracts with strong antifungal properties often have high polyphenolic content and titratable acidity. Further research is needed to isolate and characterize the polyphenolic chemicals found in the studied plant species, with the goal of encouraging their usage in agriculture to minimize fungicide treatments. Our research aimed to provide a green alternative to synthetic fungicides by identifying the optimal extract for use as a phytofungicide to manage crop diseases. These findings can be used in future investigation for the development of new potential antifungal compounds.

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Conflicts of Interest: The authors declare no conflict of interest.

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