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The Invitro Inhibitory Effects of Some Plant Extracts on Conidial Growth of The Phytopathogenic Fungus (*Fusarium oxysporum*)

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ABSTRACT

Fusarium wilt is a significant disease of vegetables that affects the tomato crop and significantly reduces productivity and is the most severe, and worldwide terrible disease. Classified on morphological criteria, pigmentation on PDA, sporulation and spore shape, the pathogenic fungus isolated from the infected tomato plant in the current investigation was identified as *Fusarium oxysporum F. sp. lycopersici*. Five extracts of the plant species *Zingibire officinale, Melia azedarach, Nerium oleander, Ocimum basilicum* and *Allium sativum* were tested *in vitro* for their fungitoxicity against the pathogen under consideration using the poisoned food technique with different concentrations (20,40,60 and 80%). The purpose of this research is to provide a safe alternative to synthetic fungicides, in comparison to the control, all of the phytoconstituents evaluated substantially decreased pathogen mycelial formation. Nevertheless, among the five plant extracts that were determined *Allium sativum* was significantly superior over other treatments and recorded (95.85%), *Ocimum basilicum* (93.15%), *Nerium oleander* (92.89%) followed by *Melia azedarach, Zingibire officinale* recorded (55%), (43%) separately.

Keywords: Fusarium Wilt, Pathogenic Fungus, Plant Extract, Poisoned Food Technic, Growth Inhibition.

1. Introduction

Tomatoes (*Lycopersicon esculentum* Mill.) are one of the most expansively grown and important Solanaceae vegetable crops. It is a vital crop for human nutrition because of its tangy fruit flavor and nutritional value Salads, soups, sauces, purees, and other forms of delectable cuisines, tomatoes are a commonly consumed fresh vegetable crop. Tomatoes come in first place among the 29 main fruits and vegetables in terms of their proportional contribution to human nutrition. Also they are positioned as a significant farmed agricultural plant by supplying crucial amino acids, vitamins such as Vit. C, and minerals, comparable to other Solanaceae plants such as potato, eggplant, pepper, and tobacco, and so on[1]. Moreover, has also been thought to be a vital fruit for cancer prevention and treatment because of its high vitamin C content and antioxidant compounds such as lycopene.

Tomatoes are susceptible to various diseases at all phases of their development, identified 24 fungiform, 7 bacterial, 7 nematodes, 10 viral, and 3 viroid infections in tomatoes; among them, *Fusarium spp* is the most diseased fungi that cause severe damage. However, fungi-induced diseases are the most common[2]. Fusarium wilt is one of the furthermost frequent and stern tomato diseases. It is caused by *Fusarium oxysporum* (*F. sp.*) *lycopersici* and causes substantial losses, outstandingly in sensitive varieties and under favorable climatic circumstances[3]. The primary symptom of the plant infected by *Fusarium spp.* the appearance of wilting, especially of mature plants, and seedlings; older leaves wilt due to the fungus, withering and becoming yellowing[4]. On one side of the plants leaf yellowing may happen, and over time, the majority of the leaves may turn yellowish, wilt and cause plant mortality. Fungal colonization causes the host plant's xylem to block and break down, which causes signs of wilt disease, including leaf withering and yellowing also plant mortality[5]. This phytopathogen fungus does have the potential to inflict severe losses through vascular disease[6]. Fusarium wilt of tomato is managed utilizing by using chemical fungicides However, these chemical fungicides infrequently cause fungi to acquire resistance to them, tend to linger in the environment for a long time, and are not biodegradable.

It has also been demonstrated that plant pathogenic fungi can evolve in response to fungicide management through mutations, leading to resistance and a reduction in the efficiency of fungicides[7]. Chemical control strategies were used in order to

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avoid plant infections. Consequently, in furtherance of managing the majority of plant infections, agronomic techniques, biological control, cultural practices, and chemical control have been established. Using synthetic fungicides for chemical control is the most widely used agricultural strategy since it is adaptable and simple to implement[8].

Much research explores environmentally friendly methods to prevent modifications in environmental dynamics and harm to both human and animal health. Plant-derived ingredients, such as natural essential oils and botanical extracts, have been examined as alternative disease-control approaches with an emphasis on their potential more environmentally and consumer-safe or to be effectively incorporated into integrated pest management systems[9]. The use of biodegradable and diverse materials, such fresh plants extracts from various parts of plants, has gained significant attention in plant disease management during the last three decades. Due to the high expense of chemical pesticides and their potentially harmful side effects[10]. Several researchers have investigated how Fusarium species influenced various plant extracts[11].

2. Methods and Materials

This investigation was carried out in a laboratory environment at the private sector in the Plant Pathology branch, Zanko Lab in Allai-bazian Sulaimani province from April to June 2021, to evaluate the Antifungal activity of plant extract of *Alium sativum*, *Nerium oleander*, *Ocimum basilicum* L, *Zingiber officinale*, and *Melia azedarach.*

2.1 Aqueous extract preparation

Chinaberry tree, sweet basil, and Nerium leaves were harvested in the local garden, Ginger and Garlic were obtained from the local market (Table 1). Different parts of the plant were aqueous extracted and tested against *Fusarium oxysporum.* Before being maintained in the laboratory for future investigations, the samples were cleaned of any foreign materials such as stones, sand, and dust. 100g of fresh plant samples (Garlic, Ginger] from each species were chopped and mixed with 100 ml of sterilized distilled water (1:1w/v) in an electric grinder. The plant extract was separated by centrifugation at 6000 rpm for 15 minutes at 4C after being initially filtered across a sterilized dual-coated gauze textile[13]. The suspension was transferred and used as 100% stock solution after being filtered along with Whatman No. 1 filter paper and kept in the fridge. In order to achieve concentrations of 20%, 40%, 60%, and 80%, the crude extracts have been diluted further with sterilized distilled water and stored for antifungal properties. For each Chinaberry, Sweet basil, and Nerium, 100g of the plant leaves were dried in the shade for 15 days to prepare dehydrated powder, and then it was crushed in a tissue grinder to a fine powder. Plant leaves were crushed into a dry powder, hanging in a medium, separated, and the suspension was stored at 4°C in impenetrable bottles for future investigation[13].

Table 1: Source of the tested plants.

No.	Common name	Scientific Name	Family	Used Part
1	Chinaberry tree	*Melia azedarach*	Meliaceae	Leaf
2	Garlic	Allium sativum	Amaryllidaceae	Bulb
3	Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
4	Nerium	*Nerium oleander*	Apocynaceae	Leaf
5	Sweet Basil	*Ocimum basilicum*	Lamiaceae	Leaf

2.2 Isolation and Identification of Fungi

To isolating the pathogens, the diseased tomato plant parts were chopped into slight sections and surface sterilized with 1% sodium hypochlorite solution for 2 minutes. After being thoroughly washed in sterile water, five pieces were positioned in Petri dishes (9mm) in diameter that contain sterilized potato dextrose-agar (PDA) media that had been treated with penicillium at 100.000 units per liter and streptomycin at 1g per liter. The dishes were incubated for a week at 26±2. The pathogens were identified in conformity with the basis of spore forms, cultural characteristics, morphological physiognomy, and microscopic characteristics according to criteria/keys described by[14,15]. The organisms were identified as *Fusarium oxysporum*. Pure cultures of the fungi were maintained by placing them in the refrigerator at 4 °C[16]

2.3 In vitro evaluation of Antifungal activity of plant extracts

Poisoned Food Technique (PFT)[17] adopted as laboratory procedures for specifying the effectiveness of aqueous plant extracts. Prepared the culture medium Potato Dextrose Agar (PDA) by taking 39 g of the culture medium with a liter of water according to the manufacturer's recommendations. Sterilized the medium with an Autoclave at a temperature of 121 °C and a pressure of 1.5 kg/cm for 20 minutes after sterilization, cool the medium to 45 C, and add Tetracycline antibiotic 250 mg/liter. Aqueous stock extracts were progressively diluted to four concentrations (20%, 40%, 60%, and 80%) by adding plant extracts to the culture medium.

2.4 Preparation of Fusarium oxysporium inoculum

A 5mm diameter sterilized cork borer was used to cut out the mycelial agar discs from the fungus one-week-old culture. Each dish contains a sterilized extract-medium mixture. The inoculated PDA dishes without extracts were utilized as a control. The plates were incubated for 7 days at 26±2ºC. The colonies' mycelial growth was measured in two directions (vertically and horizontally), and the average value of the two measurements was recorded[18]. The linear colony growth was noted at 24 hours, followed by interval measurements, and the final growth was recorded after 168 hours. The average radial growth and antifungal activity of extracts calculated as a percent growth

reduction of the test organism were calculated using the procedure[12].

$$I = [C - T]/C \times 100$$

where 'I' is growth percentage inhibited (mm), 'C' is the mean diameter of the fungus colony in control, and 'T' is the average fungal colony diameter by using plant extract.

2.5 Statistical analyses

The experiment was conducted in a completely randomized design (CRD) with three replications. The realized data were statistically analyzed according to the analysis of variance (ANOVA);-Duncan's Multiple Range Test was used for mean separation using (xlstat) of Microsoft Excel (2020) at ($p \leq 0.01$), used to identify significant variations between the results obtained in each experiment.

3. Results and discussion

3.1 In *vitro* evaluation of plant aqueous extracts on the growth inhibition of the isolated fungi in seven days after inculcation

Table 2 illustrates the growth inhibition of the identified fungi *fusarium oxysporium* by using different plant extracts *Zingibire officinale* (Ginger), *Melia azedarach* (Chinaberry tree), *Nerium oleander* (Nerium), *Ocimum basilicum* (Sweet basil) and *Allium sativum* (Garlic) at by the seventh days of incubation, the isolated fungus showed ($P \leq 0.01$) a substantial growth suppression and reduced linear growth percentage when compared to the control.

Among the plant extracts that have been evaluated showed different degrees of antifungal activity as indicated by a zone inhibition additionally, after 24hr, both *Allium sativum* and *Ocimum basilicum* recorded the highest inhibition growth rate (0.42^{d}) of *fusarium oxysporium* in the sum of five plant extracts and *Zingibire officinale* we recorded the minimum mycelial growth inhibition ($1.02^{a)}$. The activity of plant extracts was varied with exposure time for the second time *Allium sativum* recorded the maximum Fusarium growth suppression (0.68^{e}) when comparable with other plant extracts as well as for 3rd, 4th,5th,6th, and 7th day, respectively.

Overall, after 168hr of fungi inoculation, the study indicated that the growth inhibition rates of all tested extracts had antifungal effects on the underlying fungi *Fusarium oxysporium*. *Allium sativum* demonstrated maximum mycelial growth inhibition (72.77^{a}) as compared to control, followed by *Ocimum basilicum* (69.72^{b}), *Nerium oleander* (64.13^{c}), *Melia azedarach* (41.21^{d}) and *Zingiber officinale* (26.04^{e}) respectively.

The results of the current study demonstrate that the plant extract has effective antifungal properties and slowed significantly the growth of *Fusarium oxysporum* mycelium. Numerous plant extracts have been used as fungicides and have been demonstrated to inhibit pathogenic fungi from developing their mycelium and producing spores. According to[19], *Allium sativum* has been used to inhibit eighteen fungi, including *Fusarium* spp. These results are reliable with[20,21] , who have noted that *Melia azedarach* has antimicrobial solid action of other plant extracts. However, other researchers discovered that *Z. officinale* extract required maximum capability to prevent the development of *F. solani* and *F. oxysporum* in infected sweet potato tubers and tomato[22,23]. Nevertheless, the result is inconsistent with[24,25] They observed that Ginger and Moringa have strong antifungal solid against *Alternaria solani* and *Fusarium oxysporum* f. sp. *lycopersici.* The pathogen's growth and reproduction were also inhibited by that *A. sativum* extract this could be due to Allicin, an antimicrobial molecule with antifungal properties that has been documented by[26]. The anti-inflammatory and antibacterial effects of *Nerium oleander* L (Family: Apocynaceae) have been emphasized in their published studies[27]. The findings of this study showed that using sweet basil in vitro inhibited *Fusarium oxysporium* spore germination, and the percentage inhibitory zone was substantially higher than the control.[28] examined the extent to which the primary basil ingredients suppressed *F. oxysporum* growth, they determined that against *F. oxysporum*, eugenol had the most enormous rate of suppression (100%) while linalol and methyl chavicol had 26.4 and 30.3%, respectively, of inhibition.

Table 2: In *vitro* effect of different aqueous plant crude extracts on the growth inhibition rate of Fusarium oxysporum for 7 days after inoculation.

Extraction Type	24h	48h	72h	96h	120h	144h	168h	Total G. Inhibition
Zingiber officinale	1.02 ^{a}	1.96 ^{a}	2.93 ^{a}	3.78 ^{a}	4.28 ^{a}	5.69 ^{a}	6.66 ^{a}	26.04 ^{e}
Melia azedarach	0.96 ^{b}	1.68 ^{b}	2.38 ^{b}	2.99 ^{b}	3.65 ^{b}	4.46 ^{b}	5.29 ^{b}	41.21 ^{d}
Nerium oleander	0.57 ^{c}	1.02 ^{c}	1.54 ^{c}	1.58 ^{c}	2.31 ^{c}	2.77 ^{c}	3.23 ^{c}	64.13 ^{c}
Ocimum basilicum	0.42 ^{d}	0.76 ^{d}	1.17 ^{d}	1.48 ^{c}	1.79 ^{d}	2.18 ^{d}	2.73 ^{d}	69.72 ^{b}
Allium sativum	0.42 ^{d}	0.68 ^{e}	1.05 ^{e}	1.38 ^{c}	1.69 ^{d}	2.03 ^{e}	2.45 ^{e}	72.77 ^{a}

According to Duncan's multiple range test, results followed by the same small letter (a-e) within the same row are not substantially different ($P \leq 0.01$).

3.2 Determination of varied plant extract concentration effect on the Fusarium oxysporum growth inhibition

According to the findings presented in (Table 3), the fungal growth rate was significantly inhibited by various doses of plant extracts currently, also study revealed that mycelial elongation was increased in the comparison plate (control). After 24hr of incubation, the maximum growth inhibition was significantly was recorded by the highest doses of plant extracts (%80) (0.20^{e}), whereas the minimum growth suppression recorded by the lowest doses (20%) (0.47^{b}) compared to control. After 48,72,96,120, and 168hr similarly, the data illustrated no significant difference in compressions between different concentrations with the first 24hr on the mycelial growth inhibition for inoculated fungi.

Additionally, after 168hr of incubation, the largest concentration of plant extracts (80%) considerably exhibited maximum fungal growth reduction and recorded (76.78 [a]). The moderate fungal growth inhibition was recorded by the concentration (40%) (65.88 [c]) besides the minimum mycelial growth reduction (59.71[d]) was recorded by the lowest doses of plant extracts concentration (20%) for *Fusarium oxysporum* when compared to control. Ginger, Chinaberry Tree, Nerium, Sweet basil, and Garlic aqueous extracts respond differently to the test fungus depending on their tested concentrations. Similar to how the test organism responded differently to various extract concentrations to reduce Fusarium wilt in tomatoes, the best dosing may be increased using the variation in response indicated by the test organism. The different nature of their active ingredients may be the reason that aqueous extracts had a considerably higher level of fungi toxicity than ethanol extracts[29,30]. It could also be an indication of the plant material's chemical components having a higher relative water solubility. Numerous researchers have reported that uses of aqueous plant extract with diferent concentrations suppress the mycelial growth of various plant pathogenic fungi.[31,32] They found the uses of *Moringa oleifera*, *Jatropha caucus*, *Manihot esculenta*, and *Senna alata* at different concentrations suppressed the pathogenic potentials of four different Fusarium species.[33] investigated the effect of *Azadirachta indica* and *Tagetes minuta* on the development of *Fusarium oxysporum* at varying concentrations. These results are in agreement with[34] when using *Oxalis corniculata* , *Ocimum gratissimum*, *Tithonia diversifolia*, *Azadirachta indica*, *Kaempferia galangal* ,and *Zingiber officinale* against *fusarium oxysporium* the results indicated that plants extracted at varying concentrations from 3.125% to 25% considerably inhibited the growth of the tested fungus. The current study's findings are following those of[34] , who found that plant extracts of aromatic ginger and wild basil had fungi toxic characteristics in contradiction of five pathogenic fungi (*Alternaria brassicola*, *Colletotrichum capsici*, *F. oxysporum*, *Rhizoctonia solani*, and *Sclerotinia sclerotiorum*) while examined in a research lab condition used at 500 and 1000 µg/ml.[35] who discovered that when concentration rate of plant extract multiplied , the rate of mycelia growth inhibition reduced. Additionally, 100% aqueous neem *Azadirachta indica* leaf extract completely prevented *Fusarium* sp. spore germination. The growth of *C. gloeosporioides* was reduced by 39.99% by the aqueous extract of *Zingiber officinale* at a concentration of 5%[36].

Table 3: Efficacy of various plant extracts (In vitro) at different concentrations on mycelial growth inhibition of Fusarium oxysporum.

Concentration	24h	48h	72h	96h	120h	144h	168h	G. Inhibition
0	2.10 [a]	3.42 [a]	4.84 [a]	5.29 [a]	6.15 [a]	7.67 [a]	9.00 [a]	0.00 [e]
20	0.47 [b]	0.96 [b]	1.41 [b]	1.90 [b]	2.42 [b]	3.05 [b]	3.63 [b]	59.71 [d]
40	0.36 [c]	0.70 [c]	1.19 [c]	1.61 [bc]	2.08 [c]	2.56 [c]	3.07 [c]	65.88 [c]
60	0.25 [d]	0.56 [d]	0.91 [d]	1.34 [cd]	1.70 [d]	2.15 [d]	2.56 [d]	71.50 [b]
80	0.20 [e]	0.46 [e]	0.72 [e]	1.05 [d]	1.37 [e]	1.71 [e]	2.09 [e]	76.78 [a]

According to Duncan's multiple range test, results followed by the same small letter (a-e) within the same row are not substantially different ($P \leq 0.01$).

3.3 Correlation between extraction type and various concentrations on mycelial growth inhibition percentage (MGI%)

(Fig. 1) Illustrated that all plant extracts suppressed the redial growth of the fungi with different levels of efficiency at different concentrations. With an increase in the level of plant extract, the percentage of inhibition climbed dramatically. (20%) of *Allium sativum* demonstrated efficacy in reducing the colony formation of the fungus *Fusarium oxysporum*, even at the lowest concentrations were recorded up to %85 and followed by *Ocimum basilicum* (78.26%), *Nerium oleander* (66.89%) when compared with control ($p \leq 0.01$). *Melia azedarach* and *Zingibire officinale*, on the other hand, also recorded a minimum percentage of fungal growth inhibition below (50%) were recorded (43.19%) and (23.85%) for both Chinaberry tree and Ginger, respectively. Similarly the mycelial growth of *F. oxysporum* was decreased by (29.70,49.78,75.48,85.56 and 88.89%) consecutively when plant extract concentration reached (40%) for *Zingiber officinale*, *Melia azedarach*, *Nerium oleander*, *Ocimum basilicum* and *Allium sativum* separately. Additionally, after reaching the concentration from (40%) to 60(%) of the different plant extracts were suspended in the media did the phytopathogen's growth become noticeably inhibited. Clearly, the data of growth inhibition percentage in this position was very remarkable when *Nerium oleander* approached (75.48%) to (85.37%) when currently both of *Ocimum basilicum* and *Allium sativum* marked there is no noticeable difference observed between the inhibitory effect at the concentration (60%) were recorded (91.63%) and (92.74%) respectively.

The concentrations frequently correlated with the rate of mycelial growth suppression. A highly effective inhibition percentage of plant aquatic extracts was recorded in the concentration (80%) where *Allium sativum* significantly inhibited mycelial growth of isolated fungi and recorded the maximum growth inhibition percentage (%95.85) followed by *Ocimum basilicum* (93.15%) and *Nerium oleander* (92.89%) as well as leaf extract of *Melia azedarach* effects also suppressed fungal growth by up to (55%) when comparable with *Zingibire officinale* was reduced mycelial growth but not reached to (50%) recorded only (43.15%) of fungal suppression percentage. These various types of extracts had varying degrees of antimicrobial activity and also had correlations between concentration levels. The comparative differences were discovered to differ among the tested extracts. As the concentration of the extracts increased, a significant increase in the *F. oxysporum* inhibition potential was observed also effects on the liner growth of tested fungi. It was reported that both *Alternaria alternata* and *A. longipes* caused linear growth reduction by using the bulb extracts of *Allium sativum* and significantly inhibited mycelial growth[37,38] fourteen plant

extracts were examined for their ability to inhibit the growth of *A. burnsii*, the pathogen responsible for the cumin blight, the garlic cloves extract was found to be the most potent bio fungicide on the linear growth inhibition percentage. Our findings are consistent with[39,40,41] reportedly inhibited the growth of the mycelium by 78.5 and 73.2% respectively, when used the extracts of *C. gigantea* and *A. indica* when used against *F. oxysporum*.[42] investigated the effectiveness of several plant crude extracts and observed that *Hypericum triauetrifolium* ethanolic and water extracts in different concentrations were more efficient than other plant extracts against the linear development and mycelial growth inhibition percentage of *Ascochyta rabiei* and *Fusarium oxysporum. In vitro* fungal mycelial growth was considerably inhibited by all extract concentrations, this, finding is in the same line with[43], which utilized various extract dosages and recorded different value inhibition fungus mycelial growth when used E. *globulus*, *E. sativa*, and *T. foenum-graecum* against *F. oxysporum* f. sp. *melonis* and inhibited by 69.3, 66.1, and 62.8%, respectively. The fungicidal properties of *A. sativum* against *Penicillium oxalicum*, *F. solani*, *M. phaseolina*, *Botryodiplodia theobromae*, *F. oxysporum*, and *Aspergillus niger* were assessed by[44] when used 20% of concentration, *Syzygium aromaticum* and *A. sativum* completely inhibited the mycelial growth of *A. niger*.[43] noticed that in the lowest concentrations, 3.125% of aromatic ginger and wild basil both showed effectiveness in reducing the colony growth of *Fusarium oxysporum* by up to 69% and 65%, respectively.

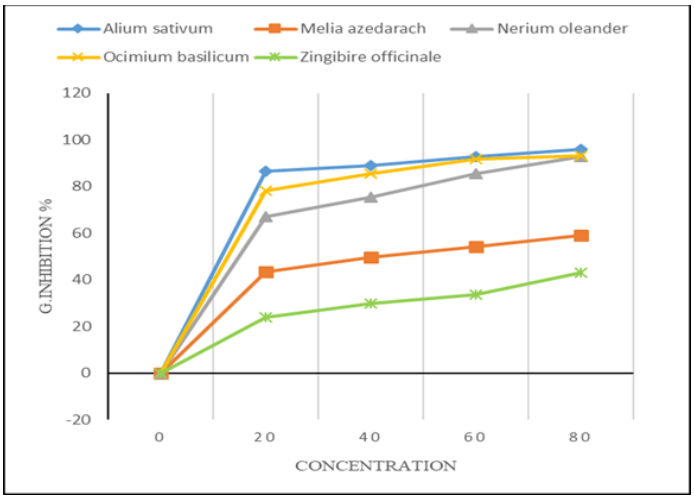

Figure 1: Effect of different concentrations of Allium sativum, Ocimum basilicum, Nerium oleander, Melia azedarach, and Zingiber officinale on mycelium growth inhibition percentage of F. oxysporum.

Conclusions

These findings indicate that *Allium sativum*, *Ocimum basilicum*, *Nerium oleander*, *Melia azedarach*, and *Zingiber officinale* of the evaluated botanical extracts have antifungal properties against *Fusarium oxysporum*. Additionally, different plant extracts may include different antifungal properties or compounds, which might be contributing to the differences in their inhibitory effects. We used several botanical extracts to be investigated as safe and environmental approaches alternatives to control soil-borne fungus in the greenhouse. We had important roles in biologically based management strategies for control of Fusarium wilt. The result suggests the application of botanical extracts has minimum and cost-effective and non-hazardous in agro-ecosystem.

Conflict of interests

None.

Author contribution

All writers contributed equally to the study; the first author mostly worked on the writing and practical aspects scientifically, while the second and third author oversaw and revised the work.

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