https://doi.org/10.26682/ajuod.2022.25.2.1
Journal of University of Duhok., Vol. 25, No.2 (Agri. and Vet. Sciences), Pp 1-8, 2022

ANTIFUNGAL ACTIVITY OF SOME PLANT EXTRACTS AGAINST FUNGUS FUSARIUM OXYSPORUM

BAREEN SDQI SHAREEF, ALI SAMI ALI, SHREEN GHAEB ALI and IBRAHIM ESSA TAHER
Dept. of Plant Protection, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region-Iraq

(Received: February 28,2022; Accepted for Publication: September 8,2022)

ABSTRACT

In vitro, study was conducted to evaluate the efficiency of *aqueous* leaf extracts for each of *Nerium oleander* , *Eucalyptus camaldulensis* and *Myrtus communis* at concentrations (0, 20, 30, and 50)% on the mycelial growth and sporulation of *Fusarium oxysporum* . The results showed that all extracts gave considerable inhibition of mycelial growth of the pathogen. The greatest inhibitory effect 31.16% was found when used *Azadirachta indica* extract.

KEY WORD: **Plant extracts, *F. oxysporum* , Oleander, River red gum, and Neem.**

INTRODUCTION

Fusarium oxysporum is a soil-borne ascomycete fungus that affects various plant hosts around the world causing major economic losses (Siddiqui *et al.*, 2016 and Joshi, 2018), particularly at the low pH soil, high humidity, and an appropriate host (Nasrin *et al.*, 2018)

Chemical control of this pathogen can reduce the risk of disease severity and may increasing crop yield, but continuous application of these chemicals may be harmful a to human health and environment (Kamalakannan *et al.*, 2004 ; Kumar *et al.*, 2007), in addition to increasing fungal population tolerance, and excessive pesticides also has an great damage on soil microorganisms (Son *et al*, 2018).

Recently, several studies found that plant extracts have a rescannable effect on most pathogenic fungi including *F. oxysporum* through modifying plant metabolism, such as accumulation of phenolic biosynthesis enzymes, antioxidant defense enzymes, plant extracts can manage various fungal infections(Ramaiah and Garampalli, 2015 ; Dabire *et al.*, 2016).

Antifungal secondary metabolites produced by plants also included flavonoids, phenolic glycosides, unsaturated lactones, sulfur compounds, saponins, cyanogenic glycosides, glucosinolates, and tannins (Vinale *et al.*,2014).

Oleander extract has antibacterial, anti-inflammatory, antinociceptive, and anticancer depressant properties that inhibit the mycelial growth of a pathogen (Ali *et al.*, 2008 ; Isaac and Abu-Tahon, 2014). Oleander composition also consist of proteins, carbohydrates, triterpenoids, aglycone oleandrigenin, glucose, resin, paraffin, tannins, and an essential oils (Zibbu and Batra, 2012).

Gupta and Bansal (2003) reported that eucalyptus leaf extracts have been shown to reduce the mycelial growth of *F. oxysporum*. Various compounds, including nimbidin, nimbin, and salannin, have been linked to the bioactivity of neem extracts, with azadirachtin

ali.sami@uod.ac

being the most important antifungal compound (Lale and Abdulrahman, 1999 ; Aboellil, 2007). The current work aimed to evaluation the activity of *Nerium oleander* , *Eucalyptus camaldulensis*, *Myrtus communis and Piper nigrum* extracts against *F. oxysporum in vitro.*

MATERIALS AND METHODS

Preparation of plant extracts

Leaves of oleander (*Nerium oleander* L., Apocyanaceae), river red gum (*Eucalyptus camadulensis* Dehnh., Myrtaceae), and neem (*Azadirachta indica*, Meliaceae) were collected from the fields of College of Agricultural Engineering Sciences. Black pepper (*Piper nigrum*, Piperaceae) was purchased from local markets in Duhok city. Samples were washed with distilled water and dried separately at 45 o C for 72 hrs.

The leaves where grinded and using distilled water as a solvent; (25) gm of each plant leaves powder were immersed in 100 ml of distilled water in beakers 500 ml and left for 12 hrs. the suspension was filtered by three layers of gauze and centrifuged at 3000 RPM for exactly one hour to separate the small residues (Shukla and Dwivedi, 2012 ; Benchimol *et al.*, 2017). The final filtrates evaporated using convection oven at 40 C for ten days.

Evaluation of different concentration of plant extracts on *F. oxysporum* activity:

Concentrations at (0, 20, 30 and 50 %) of leaves extract were prepared then 100 ml of PDA added to each concentration and shakes gently before poured in petri – dish (20 ml / per plate) with three replicates. Mycelial plugs of 5 mm of the fungus colony were cultured in the center of a plate and incubated at 25 ± 2 0 C for 7 days.

Determination of Radial Mycelium and Sporulation of *F. oxysporum*

The colony diameters for each plate was measured after full growth of control treatment. The zone of Inhibition was calculated in two directions at right angles to each another, and the percentage inhibition of mycelial growth by each extract concentration was calculated using the following formula: % of mycelial inhibition= (C –T / C)× 100

Where C and T are the mycelial growth diameter (mm) in control and treatment, respectively.

For determinate the activity of extracts against a pathogenic sporulation, fungal colony was scraped superficially on the PDA using a soft brush. The suspension was prepared and diluted to 1/100 and spores counted using a haemocytometer.

RESULTS

Effects of Extracts on the Inhibition Growth of *F. oxysporum*

The data represented in Table (1) showed that the *N. oleander* , *E. camaldnlesis* ,*M. communis* and *P. nigrum*. leaves extractions have significant effects on colony growth of *F. oxysporum*, the lowest fungal growth 3.63 cm was detected by black pepper at 20%, while the highest growth 4.35 cm showed when applied eucalyptus at 50% compared to 5.43 in control.

Table (1):- Effect of plant extracts on the colony diameter (cm) of F. oxysporum

Extracts	% Conc.			Mean
	20	30	50	
P. nigrum.	3.63 a	3.86 a	3.86 a	3.78 a
N. oleander	4.00 a	3.86 a	3.83 a	3.91 a
E.camadulensis	4.30 a	4.03 a	4.35 a	4.21 ab
A. indica.	3.73 a	3.76 a	3.70 a	3.73 a
Control	5.43 b	5.43 b	5.43 b	5.43 b
Mean	4.22 a	4.18 a	4.23 a	

* Means followed by different letters are significantly different according to Duncan's multiple range test (P = 0.01).

The results in Table (2) showed that extracts of Neem at 50% inhibited mycelial growth with 31.83 % though all extract concentrations have a significant inhibition of the pathogenic growth. Neem had the highest inhibitory mean of 31.15 % followed by oleander 27.70 % and 30.01 % of pepper. Eucalyptus produced a 22.35 percent inhibition.

Table (2):- Effect of plant extracts on the inhibition of mycelial growth of *F. oxysporum*

Extracts	% inhibition of mycelial growth of F. oxysporium			Mean
	20	30	50	
***P. nigrum*.**	33.15 a	28.48 a	28.41 a	30.01 a
N. oleander	25.46 a	28.61 a	29.04 a	27.70 a
E. camadulensis	20.85 b	26.28 a	19.92 b	22.35 ab
A. indica.	31.03 a	30.60 a	31.83 a	31.15 a
Mean	27.62 a	28.49 a	27.30 a	

* Means followed by different letters are significantly different according to Duncan's multiple range test (P = 0.01).

Effect plant extracts on the sporulation percentage of *F. oxysporum*:

The results revealed that all examined extracts showed comparable reduction in fungal spores when compared to the control. The highest spore production (4.18 $*\ 10^{5}$) was resulted by Neem at 50%, while the lowest sporulation (2.16 $*\ 10^{5}$) was recorded by oleander and eucalyptus at 20% and 50%, respectively. (Table 3)

Table (3) : -Effect of plant extracts on the sporulation % of *F. oxysporum*

Extracts	% sporulation * 10^5			Mean
	20	30	50	
P. nigrum.	3.54 ab	3.25 ab	3.00 a	3.26 a
N. oleander	3.20 ab	3.15 ab	2.16 a	2.84 a
E. camadulensis	2.16 a	3.16 ab	3.83 ab	3.05 a
indica	4.08 ab	2.25 a	4.18 ab	3.50 a
Control	5.50 b	5.50 b	5.50 b	5.50 b
Mean	3.69 a	3.46 a	3.73 a	

* Means followed by different letters are significantly different according to Duncan's multiple range test (P = 0.01).

DISCUSSION

The application of chemical compounds became hazardous to both human health and the environment. Therefore, bio-products of plant extracts could be used because the materials used were none expensive and easily prepared and applying with low concentrations (Chacón *et al*,.2021). Currently, plant extracts are an alternative methods for control soil borne pathogens because they contain a wide range of secondary metabolites that have been shown to have antimicrobial properties (Zakuan *et al.,* 2018).

Essential oils also are a complex mixture of volatile secondary metabolites known for their natural phenolic components, such as diterpenes, hydrocarbons, and monoterpenes with various functional groups, which have been studied for their antifungal activities (Ray *et al.,* 2018 and Salem *et al.,* 2016).

Antifungal effects of extracts and essential oils from numerous higher plants have been discovered that contain several active secondary metabolites for more than 80% of all Terpenoid, Alkaloids, and Phenol secondary metabolites (Newman et al. 2000 ; Osman and Mohamed 2017). Katooli et al. (2012) and Siramon et al., (2013) investigated the antifungal activity of *Eucalyptus camaldulensis* essential oil on the suppression of pathogenic fungi.

Extracts from the leaves of several aromatic plants have recently been shown to have a strong antifungal effect due to polyphenolic composition of flavonoids and phenolic acids in the soluble fractions of *E. camaldulensis* (Sabo and Knezevic, 2019). Methanolic extracts revealed the presence of protocatechoic, gallic, ellagic acids, and vanillic and protocatechoic aldehyde, as well as quercetin, erodictyol, vanillin, naringenin, naringin, kaempferol quercitr (Hassan et al., 2021). *E. camaldulensis* exhibited significant antifungal activity against *F. oxisporium*, which could be attributed to quercetin 3-O-rhamnoside, ellagic acid, caffeic acid, quercetin 3-ObD-glucuronide, acid chlorogenic, p-coumaric acid, and ferulic acid (Elansary *et al.,* 2017).

The foliage and roots of *N. oleander* were confirmed to have antifungal activity in the current studies Ullah *et al.,* (2014) claim that distinct portions have photochemical properties that prevent bacteria from growing. Yadav et al. discovered tannins, alkaloids, carbohydrates, flavonoids, and glycosides in various sections of *N. oleander*. Rajendran (2011) discovered antifungal agents myricetin and rutin in the dried leaves of *N. oleander* in a study. Siddiqui *et al.,* (2016) discovered antifungal compounds such as oleanolic acid, amyrine terpenoids, pentamethoxyflavones, and isoflavonoids in the leaves.

The effect of *Azadirachta indica* aqueous extracts on *Fusarium oxysporum* was investigated (Singh et al., 1980). In vitro fungicidal properties of neem leaf extracts were attributed by (Mahmoud *et al.*, 2011) to the presence of several antimicrobial active ingredients in neem leaves such as sitosterol, quercetin, and desactylimbin. Other researchers have attributed this activity to the presence of active ingredients such as triterpenes or limonoids such as meliantriol, azadirachtin, desactylimpin, quercetin, sitosterol, nimbin, nimbinin, nimbidin, nimbosterol, and margisine (Bhatnagar and McCormick.,1988)

Piper is one of the most well-known genera in the Piperaceae family, which is well known in culinary and traditional medicine. Plants in this genus have essential oils with a wide metabolic range (Da Silva et al., 2014). Piper species essential oils include about 270 chemicals, with mono and sesquiterpene hydrocarbons accounting for over 80 of them, followed by phenols, aldehydes, acids, alcohols, ketones, and esters (Salehi et al., 2019). Secondary metabolites found in Piper extracts have been shown to have antifungal and cytotoxic activity. Secondary Piper's compounds with biological activity including dilapiol, safrole, methylenedioxyphenyl, and myristicin (Granados-Echegoyen et al., 2015).

REFERENCES

Aboellil, A.H., 2007. Trilogy, a product of neem (Azadirachta indica) induces resistance in cucumber against Podosphaera xanthi. Research Journal of Microbiology 2 (5), 402–414.

Ali, S.S., S. Ali, S. Munir, T. Riaz (2008). Insecticidal and bactericidal effects of ethanolic leaf extract of common oleander, Nerium oleander. Punjab Univ. J. Zool., 23(1-2): 081-090

Benchimol, R. L., Sutton, J. C., Bastos, C. N., da Silva, C. M., & Dias-Filho, M. B. (2017). By-products of Piper aduncum in the control of fusariosis in black pepper plant. *Embrapa Amazônia Oriental-Artigo em periódico indexado (ALICE)*.

Bhatnagar, D. and McCormick, S.P. (1988) : The inhibitory effect of neem (Azadirachta indica) leaf extracts on aflatoxin synthesis in Aspergillus parasiticus. Journal of the American Oil Chemists'Society, 65(7): 1166-1168.

Chacón, C., Bojórquez-Quintal, E., Caamal-Chan, G., Ruíz-Valdiviezo, V. M., Montes-Molina, J. A., Garrido-Ramírez, E. R., ... & Ruiz-Lau, N. (2021). In Vitro Antifungal Activity and Chemical Composition of Piper auritum Kunth Essential Oil against Fusarium oxysporum and Fusarium equiseti. *Agronomy*, *11*(6), 1098.

Da Silva, J.K.R.; Silva, J.R.A.; Nascimento, S.B.; Da Luz, S.F.M.; Meireles, E.N.; Alves, C.N.; Ramos, A.R.; Maia, J.G.S. Antifungal activity and computational study of constituents from Piper divaricatum essential oil against Fusarium infection in black pepper. Molecules 2014, 19, 17926–17942. [CrossRef]

Dabire, T. G., Bonzi, S., Somda, I., & Legreve, A. (2016). Activité antifongique d'extraits aqueux de Cymbopogon citratus (DC) Stapf., Eclipta alba (L.) Hassk. et Portulaca oleracea (L.) contre les principaux champignons transmis par les semences d'oignon (Allium cepa L.) au Burkina Faso/[Antifungal activity of Cymbopogon citratus (DC) Stapf., Eclipta alba (L.) Hassk. and Portulaca oleracea (L.) aqueous extracts against the main seed-borne fungi of Onion (*Allium cepa* L.) in Burkina Faso]. *International Journal of Innovation and Applied Studies*, *17*(3), 804.

Elansary, H.O.; Salem, M.Z.M.; Ashmawy, N.; Yessoufou, K.; El-Settawy, A.A. In vitro antibacterial, antifungal and antioxidant activities of Eucalyptus spp. leaf extracts related to phenolic composition. Nat. Prod. Res. 2017, 31, 2927–2930. [CrossRef]

\Granados-Echegoyen, C.; Pérez-Pacheco, R.; Bautista-Martínez, N.; Alonso-Hernández, N.; Sánchez-García, J.A.; MartinezTomas, S.H.; Sánchez-Mendoza, S. Insecticidal effect of botanical extracts on developmental stages of Bactericera cockerelli (Sulc) (Hemiptera: Triozidae). Southwest. Entomol. 2015, 40, 97–110. [CrossRef]

Gupta RK, Bansal RK (2003). Comparative efficacy of plant leaf extracts and fungicides against Fusarium oxyporum Schlecht inducing fenugreek wilt under pot house condition. Ann. Biol. 191:35– 37.

Hassan, H. S., Mohamed, A. A., Feleafel, M. N., Salem, M. Z., Ali, H. M., Akrami, M., & Abd-Elkader, D. Y. (2021). Natural Plant Extracts and Microbial Antagonists to Control Fungal Pathogens and Improve the Productivity of Zucchini (Cucurbita pepo L.) In Vitro and in Greenhouse. *Horticulturae*, *7*(11), 470.

Isaac, G.; Abu-Tahon, M.(2014). In vitro antifugal activity of medicinal plant extract against Fusarium oxysporum f. sp. lycopersici race 3 the causal agent of tomato wilt. Acta Biol. Hung., 65, 107–118. [CrossRef]

Joseph B, Dar M. A and Kumar V, Global J. Biotechnol. Biochem, 2008, 3: 56-59.

Joshi, R. (2018). A review of Fusarium oxysporum on its plant interaction and industrial use. *J. Med. Plants Stud*, *6*(3), 112-115.

Kamalakannan A., Mohan L., Harish S., Radjacommare R., Amutha G., Chitra K., Karuppiah R., Mareeswari P., Rajinimala N. & Angayarkanni T. (2004). Biocontrol agents induce disease resistance in Phyllanthus niruriLinn against damping-off disease caused by Rhizoctonia solani. Phytopathol. Mediterr. 43: 187–194.

Katooli N, Maghsodlo R, Honari H, Razavi SE. 2012. Fungistatic activity of essential oil of thyme and eucalyptus against of postharvest and soilborne plant pathogenic fungi. Global J Med Plant Res 1 (1): 1-4.

Kumar, R., Dubey, N. K., Tiwari, O. P., Tripathi, Y. B., & Sinha, K. K. (2007). Evaluation of some essential oils as botanical fungitoxicants for the protection of stored food commodities from fungal infestation. *Journal of the Science of Food and Agriculture*, *87*(9), 1737-1742.

Mahmoud, D. A., Hassanein, N. M., Youssef, K. A., & Abou Zeid, M. A. (2011). Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Brazilian Journal of Microbiology*, *42*(3), 1007-1016.

N.E.S. Lale and H.T. Abdulrahman, Evaluation of neem (Azadirchta indica A. Juss) seed oil obtained by different methods and neem powder for the management of Callosobruchus maculates(F.) (Coeloptera: Burchidae) in stored cowpea, J.Stored Peod. Res.,35, 1999,135-143.

Nasrin, L., Podder, S., & Mahmud, M. R. (2018). Investigation of Potential Biological Control of Fusarium Oxysporum f. sp. Lycopersici by Plant Extracts, Antagonistic sp. and Chemical Elicitors.

Newman DJ Cragg GM, Snader KM. 2000. The influence of natural products upon drug discovery. Natural Prod Rep 17 (3): 215-234.

OSMAN, A. O. A., & MOHAMED, I. S. (2017). Antifungal evaluation of some leaves extracts and fungicide against Fusarium oxysporum f. sp. lycopersici causal agent wilt of tomato. *Asian Journal of Tropical Biotechnology*, *14*(1), 1-8.

Rajendran, A. (2011). Isolation, Characterization, Pharmacological and Corrosion inhibition Studies of Flavonoids obtained from Nerium oleander and Tecoma stans. Int. J. Pharm Tech Res. 3(2):1005-1013.

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.

Ray, A.; Jena, S.; Dash, B.; Kar, B.; Halder, T.; \\\Chatterjee, T.; Ghosh, B.; Panda, P.C.; Nayak, S.; Mahapatra, N. Chemical diversity, antioxidant and antimicrobial activities of the essential oils from Indian populations of Hedychium coronarium Koen. Ind. Crop. Prod. 2018, 112, 353–362. [CrossRef]

Sabo, V.A.; Knezevic, P. Antimicrobial activity of Eucalyptus camaldulensis Dehn. plant extracts and essential oils: A review. Ind. Crop. Prod. 2019, 132, 413–429. [CrossRef] [PubMed]

Salehi, B.; Zakaria, Z.A.; Gyawali, R.; Ibrahim, S.A.; Rajkovic, J.; Shinwari, Z.K.; Khan, T.; Sharifi-Rad, J.; Ozleyen, A.; Turkdonmez, E.; et al. Piper species: A comprehensive review on their phytochemistry, biological activities and applications. Molecules 2019, 24, 1364. [CrossRef]

Salem, M.Z.M.; Zidan, Y.E.; Mansour, M.M.A.; El Hadidi, N.M.N.; Abo Elgat, W.A.A. Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi. Int. Biodeterior. Biodegrad. 2016, 106, 88–96. [CrossRef]

Shukla, A., & Dwivedi, S. K. (2012). Bioefficacy of plant extracts against Fusarium species causing wilt in pulses. *IOSR Journal of Engineering*, *2*(1), 136-144.

Siddiqui, I., Bokhari, N. A., & Perveen, K. (2016). Antifungal ability of Nerium oleander against Fusarium oxysporum, Sclerotium rolfsii and Macrophomina phaseolina. *J. Anim. Plant Sci*, *26*(1), 269-274.

Singh, U. P., Singh, H. B., & Singh, R. B. (1980). The fungicidal effect of neem (Azadirachta indica) extracts on some soil-borne pathogens of gram (Cicer arietinum). *Mycologia*, *72*(6), 1077-1093.

Siramon, P.; Ohtani, Y.; Ichiura, H. Chemical composition and antifungal property of Eucalyptus camaldulensis leaf oils from Thailand. Rec. Nat. Prod. 2013, 7, 49–53.

Son D., Zerbo F.K.B., Bonzi S., Legrève A., Somda I. & Schiffers B. (2018). Assessment of Tomato (Solanum lycopersicum L.) Producers' exposure level to pesticides, in Kouka and Toussiana (Burkina Faso). Int. J. Environ. Res. Public Health, 15, 204. Doi:10.3390/ijerph15020204.

Ullah, N., I. Ahmed, S. Ayaz (2014). In Vitro Antimicrobial and Antiprotozoal Activities, Phytochemical Screening and Heavy Metals Toxicity of Different Parts of Ballotanigra. Bio Med Res.Int :1-9.

Vinale, F., G. Manganiello, M. Nigro, P. Mazzei, A. Piccolo, A. Pascale, M. Ruocco, R. Marra N. Lombardi, S. Lanzuise, R. Varlese, P. Cavallo, M. Lorito, L.W. Sheridan (2014). A Novel Fungal Metabolite with Beneficial Properties for Agricultural Applications. Molecules 19:9760- 9772

-

Zakuan, Z.; Mustapa, S.A.; Sukor, R.; Rukayadi, Y. Antifungal activity of Boesenbergia rotunda (temukunci) extract against filamentous spoilage fungi from vegetables. Int. Food Res. J. 2018, 25, 433–438.

Zibbu, G., A. Batra (2012). In vitro and in vivo determination of phenolic contents and antioxidant activity of desert plants of Apocynaceae family. Asian J. Pharmaceut. Clinical Res. 5 (suppl 1):76-83.