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EFFECT OF SOME PLANT LEAVES EXTRACTS ON *Fusarium culmorum* THAT CAUSE WHEAT DAMPING- OFF DISEASE

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ABSTRACT

In vitro, study was conducted to evaluate the efficiency of leaves extracts for each of *Nerium oleander* , *Eucalyptus camaldulensis* and *Myrtus communis* at concentrations 0, 0.5 , 1 , 2 and 3 % on the growth and sporulation of *Fusarium culmorum* , the cause of wheat damping- off disease . Results revealed anoticable inhibition of a pathogen mycelial growth and its sporulation when cultured on PDA mixed with different extracts particularly with *M. communis* that exhibited inhibition of mycelial growth by the rate of 30.06 %, compared to 26.31 % and 14.77 % with extracts of *E. camaldulensis* and *N. oleander* , respectively. The lowest comparable sporulation of apathogen was 12.07% recorded in colonies grown on *E. camaldulensis* extract at 0.5 % .Wheat seedlings damping –off % was reduced significantly with increasing concentrations of examined extracts.

KEYWORDS **:** Wheat damping-off **, *F. culmorum*,** Bioassay , Plant extracts

INTRODUCTION

The fungi belonging to the genus *Fusarium* are ubiquitous in soil whenever wheat is grown . They are particularly numerous and active in the rhizosphere region of wheat, especially its roots (Palazzini et al. , 2007). *Fusarium* species are causal agents of several diseases on wheat. They cause seedling blight, root and foot rot and head blight resulting in yield losses (Wiese, M.V. 1987).

Several Fusarium species have been described on wheat among which *F. graminearum, F. culmorum, F. poae* and *F. triticum* are the most frequently reported species (Parry et al., 1995). In Tunisia , *F. culmorum* has been reported as the dominant pathogen species responsible of causing wheat diseases (Gargouri et al., 2003., Kammoun-Gargouri et al., 2009).

The seeds infected with *Fusarium* may induce a loss of seed germination, a reduced emergence, a post emergence and blight of seedlings, thus causing less dense plant stand. Pesticides use as a seed treatment has been shown to be effective in reducing the seedborne inoculate of *Fusarium* (Reddy et al., 1999) , but toxic residues associated with fungicides can be problematic. The reduction in the use of pesticides, due to their harmful environment and human health side effects has resulted in increasing the interest in the use of biological control methods (Singh, U.P. and Prithiviraj, B. 1997 ; Uoti, J. 1995.).

To minimize the side effects of chemical application , many efforts have been carried out to utilize the antimicrobial activity of plant extracts. Several studies showed the importance of natural chemicals from medicinal plant extracts as a possible source of non – phototoxic, systemic and easily biodegradable alternatives (Amadioha , 1998 ; Amadioha , 2002) .

Eucalyptus camaldulensis oil has pesticidal , nemticidal and insecticidal activity , it has been reported that 22 tested bacterial strains and 11 fungal strains were instantly killed by using eucalyptus oil (Pattanaik et al. , 2002).

The toxicity of *Nerium oleander* is considered extremely high and it has been reported that in some cases only a small amount had lethal or near lethal effects. The most significant of these toxins are oleandrin and nerine, which are cardiac glycosides [Sabira Begum, et. al. , (1999)] .

Myrtus communis L. is an annual plant that has been used since ancient times for medicinal, food and spices purposes(Romani et al. , 1999). The leaves contain tannins, flavonoids such as quercetin, catechin and myricetin derivatives and volatile oils [Baytop ,1999; Romani et al.,1999].

MATERIALS AND METHODS

The extracts of three plant leaves, *Nerium oleander* (apocynaceae) , *Eucalyptus camaldulensis* (Myrtaceae) and *Myrtus communis* (Myrtaceae) were used in this work.

The leaves were collected at the beginning of Octobar in 2014 from the fields of Collage of Agriculture, University of Duhok .Leaves were washed under distilled water (D.W) and dried separately in an oven at 45 ° C for 72 h. .

Preparation of Plant Extracts:

The plant extracts were prepared in Plant Protection Department laboratory / Agricultural College / Duhok University in 2014. The leaves where crushed and milled by Electric Blender adopting method (Rios et al., 1987), and using distilled water as a solvent, at the rate of 1: 20 (w : v) for each of leaves powder (L,P) and D.W. respectively, thus 300 gm of each plant leaves powder were added to distilled water and completed the final volume to 6 litter (the suspension divided on six – 1 liter size Beaker). The beakers left in a rocking device horizontal type at medium speed and left the sample to settle for half an hour , then nominated by three layers of gauze . The nominated suspension re-nominated by using a centrifuge with fast 3000 RPM for exactly one hour to separate the small plankton (Al – Thahab , 1998 and Al–Zorfi , 2003). Then the filtrates evaporated using convection oven at 40°C degree for ten days , to dried the extracts and obtained heavily abstract of 52 gm.

Isolation, purification and pathogenicity test of *F. culmorum*

The fungus *F. culmorum* was identified using taxonomic key as descrited by Dosmasch and Gams, 1980 , and kept under the No. FPZ 68. The parts were incubated on PDA culture at 25 ± 2 ^{0}C for one week. After growing, the fungus was purificated and identified .

The inoculum of the fungus was mixed with soil previously sterilized by formalin 1%. Then , Wheat seeds was sterilized (surface disinfection) by a 2 % Sodium Hypochlorite (NaOCL) and washed by distilled water then each five seeds were planted in a small pots filled with the previous mixture of sterilized soil and fungus inoculum (200/ pot) , and replicated three times , to calculate the germination percentage .

Evaluation of different concentration of plant extracts on *F. culmorum* a ctivity:

Concentrations of *Nerium oleander*, *Eucalyptus camaldulensis* and *Myrtus communis* leaves extract were as prepared as (0.5 , 1 , 2 and 3) % in addition to control treatment (0.0) then the PDA culture added to each concentration to complete the size to 100 ml. The culture was well shaked , then poured inside a sterilized petri – dish (20 ml / per dish) with three replication .

For each petridish , one disk of 5 mm diameter of the fungus colony (cut by a cork borer) were added and incubated at 25 ± 2 ^{0} C for seven days . The colony diameters for each petridish was calculated and compared with control.

The zone of Inhibition was measured in two directions at right angles to each other , and the percentage of mycelial growth inhibited 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 .

Fungus inoculums preparation and soil treatment :

The *Fusarium culmorum* was isolated from wheat seedlings , collected from Agricultural College fields was multiplied on PDA media .

After growing a concentration of 1× 10^5 spore / ml suspension was prepared , for each 800 gmof sterilized soil ,25 ml of suspension (concentration 1 × 10^5) was added , then well mixed.

Mixing of leave extracts with the soil:

The soil that previously inoculated with fungus suspension at 1 × 10^5 spore / ml was divided to 5 parts. Each part was subdivided to other 5 parts , each contain 800 gm and kept in a small pots.

To evaluate the effect of plant leave extracts on the seedlings , ten ml of leaves extract suspension / replicate in five concentrations (0 , 0.5, 1, 2 and 3 %) were added to the pot soil with three replication .The control treatment (0.0 %) has included the addition of distilled water . Then for each pot , ten wheat seeds were planted , irrigated and kept under laboratory conditions .The damping – off percentage was measured after 30 days of planting.

Qualitative analysis of High Performance Liquid Chromatography(HPLC) profiling:

The analysis used to state the chemical profiling of the crude examined extracts using HPLC system at College of Medicine , University

of Salaheddin, Erbil type L C - 6A provided by Shimadza , Germany.

Solvents of n- hexane , methanol , n- heptane with ethyl acetate and acetic acid were mixed at the rate of 2:5:5 to make sure that the lipid and fatty acid compound have been flushed away to avoid clog or stuck of column in the HPLC . Ten grams of each methanolic extract was mixed with 10 grams of silica and forward to the column chromatography 20 µl of diluted extract sample was injected to the HPLC and the separation was realized into a C 18 reverse phase HPLC column type ODS (260 × 4.6 mm) with a low pressure gradient.

The folw rate of the solvent used was 1.0 ml/ min., starting at 10 % methanol , 15 % acetonitrite , 75 % deionised water and continue with increasing polarity of the solvent .

Statistical analysis

The data was analysed according to the factorial experiment arranged in Completely Randomized Design with three replications and Duncan Multiple Range Test were used to compare between treatments at 1% probability level.

RESULTS AND DISCUSSION

Inhibition percentage of mycelial growth of F. culmorum as affected by three plant leaves extract

The data represented in Table (1) showed that the *N. oleander* , *E. camaldnlesis* and *M. communis* leaves extractions have significant effect on inhibition percentage of *F. culmorum* mycelial growth .

Table (1) : Effect of plant extracts on the inhibition mycelial growth of ***F. culmorum***

Extracts	% inhibition of mycelial growth of *F. culmorum*				Mean
	0.5%	1%	2%	3%	
N. oleander	11.35 d	7.51 d	15.64 cd	24.59 b	14.77 b
E. camaldulensis	27.34 b	29.17 ab	25.46 b	23.29 bc	26.31 a
M. communis	23.74 bc	28.49 ab	36.51 a	31.51 ab	30.06 a
Mean	20.81 b	21.72 ab	25.87 a	26.46 a	

* Means followed by different letters are significantly different based on Duncan's Multiple Range test (P=0.01).

The higher and considerable inhibition (30.06% and 26.31 % of the mycelial growth was detected when used extracts of *M. communis* and *E. camaldulensis* ,respectively compared to the lowest fungal suppression 14.77% with an extract of *N. oleander* . However, the higher inhibitory effect 36.51% was recorded by *M. communis* at 2%. *N. oleander* produced weak inhibition of 7.51 % at 1%.

Effect of three plant leaves extracts on the inhibition of sporulation percentage of *F. culmorum*:

The data present in Table (2) , indicated that the sporulation percentage of *F. culmorum* varied according to the extract types and concentrations used.

It is clear , that the sporulation percentage increased gradually with increasing the extract concentrations.

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Table (2) : Effect of plant extracts on the inhibition sporulation % of ***F. culmorum***

Extracts	% inhibition of sporulation of *F. culmorum*				Mean
	0.5%	1%	2%	3%	
N. oleander	13.73 e	18.47 bcd	20.37 ab	21.17 a	18.43 ab
E. camaldulensis	12.70 e	17.67 cd	20.27 ab	21.13 a	17.94 b
M. communis	16.83 d	19.93 abc	19.40 abc	21.13 a	19.32 a
Mean	14.42 c	18.69 b	20.01 a	21.14 a	

*Means followed by different letters are significantly different based on Duncan's Multiple Range test (P=0.01).

Apparently, the pathogen,s sporulation was effected remarkably with increasing of the extract concentrations , since spores density was reduced by more than 21% when used extracts at 3% compared to 19.4- 20.37% at 2% (Table 2). *M. communis* was the best when reduced the fungal sporulation by 19.32% and the minimum unhibitory concentration was 0.5% for both *N. oleander* and *E. camaldulensis* which inhibited the sporulation by 13.73 % and 12.7% respectively.

Effect of three plant extraction on wheat seedlings damping- off percentage:

The results in (Fig. 1) showed that he highest daming –off percentage was scored on the seeds planted in a soil treated with *N. oleander* leaves extraction (51.33 and 35.33 %) for pre and post germination , respectively compared with 43.33 and 23.33 damping- off percentage when the seeds planted in soil treated with *E. camaldulensis* which not significantly differ from those scored by *M. communis.*

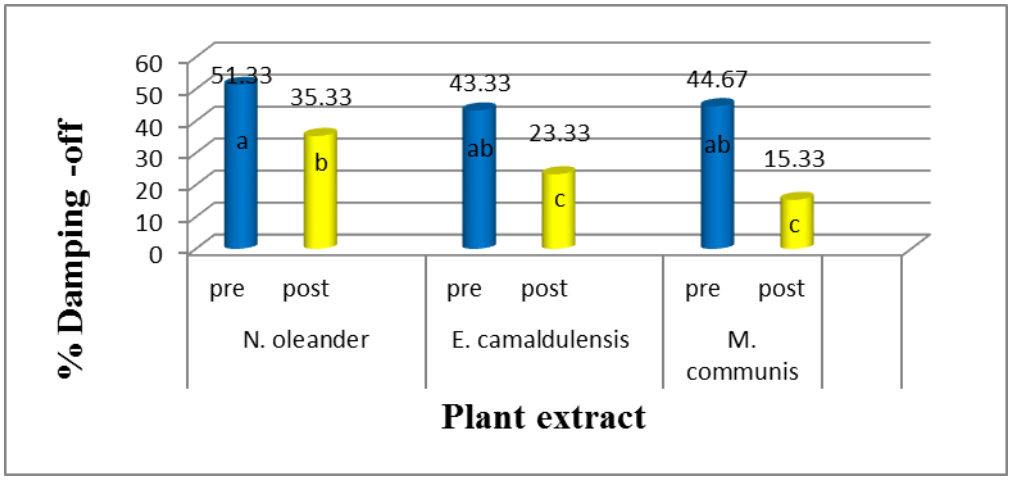

Fig. (1): Effect of plant extracts reduction of damping- off % of wheat seedlings.

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According to th results in (Fig. 2) , there were a significant differences among the seedlings damping –off % depending on differ concentrations of the three plant extracts. The damping –off % was decreased by increasing the concentrations of leaves extrats. It seems that , the leaves extract have an enhancing effect on seed germination as compared to control treatment. The pre damping- off % for seeds planted in soil treated with 0.5 concentration was (51.11%) and not differ from the damping – off % resulted by 1 concentration (Fig. 2).

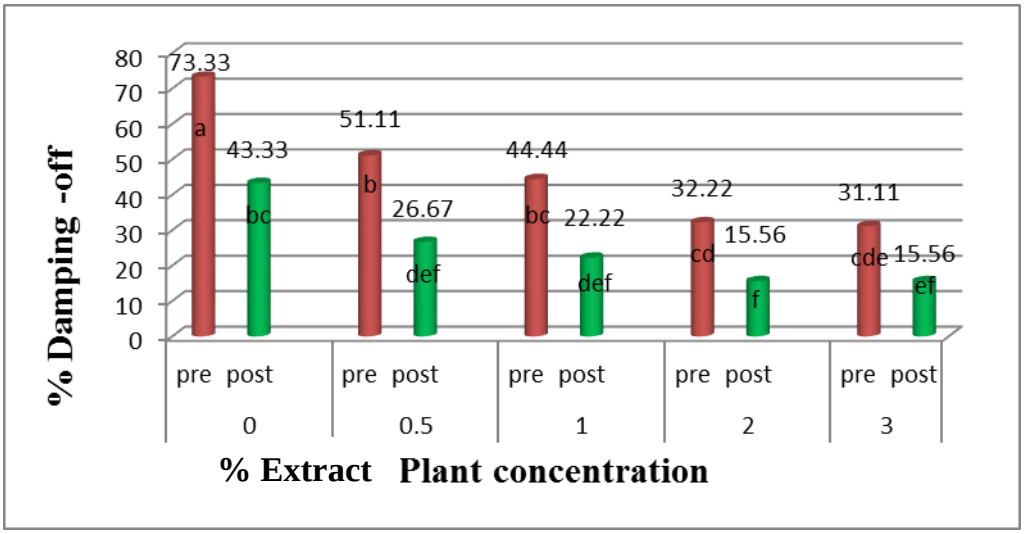

Fig. (2): Effect of extract concentrations on the reduction of damping –off of wheat seedlings.

The lowest reduction in percentage of post germination damping – off (3.33 %) was recorded for seeds planted in soil treated with *E. camaldulensis* leaves extract at a concentration 3% (Table 3) which not significantly differ from those scored by 1 and 2 % concentrations as 13.33 % for both of them , respectively , compared to 43.33 , 23.33 and 36.67 % for seeds planted in soil treated with *N. oleander* extract at 1 , 2 and 3 concentrations , respectively as a highest reduction in the same character..

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Table (3) : Pre and post damping- off percentage of wheat seeds planted in soil treated with plants extractions.

Extracts	Extracts concentration %									
	0		0.5		1		2		3	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
N. oleander	73.33 a	43.33 bcd	53.33 abc	30.00 cdefg	43.33 bcd	43.33 bcd	40.00 bcde	23.33 defg	46.67 abcd	36.67 bcdef
E. camaldulensis	73.33 a	43.33 bcd	36.67 bcdef	43.33 bcd	40.00 bcde	13.33 efg	30.00 cdefg	13.33 efg	36.67 bcdef	3.33 g
M. communis	73.33 a	43.33 bcd	63.33 ab	6.67 g	50.00 abcd	10.00 fg	26.67 cdefg	10.00 fg	10.00 fg	6.67 g

* Means followed by different letters are significantly different based on Duncan's Multiple Range test (P=0.01).

Jumaa and Ibrahim , (2011) mentioned that the wheat seeds germination was enhanced by *E. camaldulensis* leaves extractions at low concentration (0.5 %) , and decreased by increasing the concentration to 1 , 3and 5%. Al – Sultani , (2000) ; Vaugh and Boydston , (1997) and Weston (1996) , mentioned that plant extracts have an inhibition effect on wheat seed germination which related to their content of phenols and alkaloids.

Qualitative Analysis of High Performance Liquid Chromatography (HPLC) Profiling

The differences among the plant leaves in their inhibitory ability is due to the presence of active materials in their structure (table 4) , that the *M. communis* has the highest percentage of Alkaloids and Flavonoids as 15.2 and 12.25 % , respectively, compared to 1.76 and 4.62, 0.00. and 3.6 % for *N. oleander* and *E. camalduulensis* , respectively.

Table (4): Qualitative phytochemical analysis of the three plant leaves extracts

Active material % / Extracts	Saponins	Glycosides	Alkaloids	Flavonoids
N. oleander	-	1.02	1.76	4.62
E. camaldulensis	3.1	2.2	-	3.6
M. communis	3.6	1.03	15.2	12.25

Sarkar et.al. (1996) mentioned that the effective potential of *M. communis* leaves extract may be due to their higher content of Flavonoids which have the ability in reducing where affect later on carbohydrate metabolism process and reducing the energy units (ATP) . Al- Zohyri (1982) also indicated that the *M. communis* leaves and flowers contain Glycosides , Saponines and Phenols.

We conclude that wheat damping – off caused by *Fusarium culmorium* may be reduced using plant extracts of *N. oleander , E. camaldulensis* and *M. communis* . This results were supported by in Vitro significant inhibition of fungal growth and its sporulation.

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شیانا گیراوییِن بە لگیین رووە کی بۆراگرتنا F. culmorum کوبیتە هوکاری مرنا نە مامییِن کە نمی

پوختە

تاقیکرنا ژیانی ل تاقیگە هی هاتە کرن بۆ دیارکرنا شیانا گیراوییِن بەلگیین رووەکی هەر ئیك ژ Nerium oleander و Eucalyptus camaldulensis و Myrtus communis بچریا (چنە 2,1, 3 ,)ل سەر گەشەکرن و دوورستکرنا سپوراتا نساخیی رووەکی یاکەرووی F. culmorumکو دبیتە ئەگەری مرنا نەمامییِن گەنمی.

ئەنجاما تاقیکرنی دیاردکەن کو راگرتنەکا بەرچاف هەبوویە بو گەشەکرنا مایسیلیومی وچیکرنا سپوراتا ل دما چاندنا وان ل ناڤەندی خۆراکی PDA تیکەل دگەل گیراوییِن جودا جودا و بتایبەت M. communis کو دیارکر بیتە ئەگەری راگرتنی بریژەیا 30.06 % بەراوورد دگەل 26.31 و 14.77 % گیراوییِن Eucalyptus camaldulensis و Nerium oleander ل دیف ئیك .

کیمترین ریژا چیکرنا سپوراتا کەرووی بریژا 12.07 % ل کەروویِن گەشەکرنی ل سەر گیراوییِن E. camaldulensis بچریا 0.5 % . ریژا مرنا نەمامان ل گەنمی کیم ببون بریژەکا بەرجاف دگەل زیدەکرنا چرییا گیراوییِن رووەکی ل تاقیگەهی .

تاثير بعض مستخلصات الاوراق النباتية في الفطر *Fusarium culmorum* المسبب لمرض سقوط (موت) بادرات الحنطة

الخلاصة

تم اجراء الاختبار الحيوي مختبريا لتقييم كفاءة مستخلصات اوراق كل من *Nerium oleander* و *Eucalyptus camaldulensis* و *Myrtus communis* بتراكيز (0 ، 0.5 ، 1، 2 و 3) % في نمو وتبويغ الممرض *Fusarium culmorum* المسبب لمرض سقوط بادرات الحنطة . اثبتت النتائج تثبيطا واضحا للنمو المايسيلومي للمسبب وتبويغيه عند زراعة على الوسط الغذائي PDA المخلوط مع المستخلصات المختلفة وخاصة *Myrtus communis* الذي اظهر تثبيطا لنمو الممرض بنسبة 30.06 % مقارنة مع 26.31 % و 14.77 % لمستخلصات اوراق *Eucalyptus camaldulensis* و *Nerium oleander* على التوالي .سجل اقل نسبة تبويغ للممرض والبالغة 12.07 % في مستعمراته النامية على مستخلص *Eucalyptus camaldulensis* بتركيز 0.5 % . اختزلت نسبة سقوط بادرات الحنطة بشكل معنوي مع زيادة تراكيز المستخلصات المختبرة .