تأثير مستخلص أوراق نبات الكاربس *Conocarpus erectus* L على الفطرين *Alternaria solani* و*Ulocladium botrytis* المعزولين من جذور نبات خيارالقثاء *Cucumis melo* L.

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الخلاصة :-

أختبر تأثير المستخلصات المائية الباردة والحارة والميثانولية والايثانولية لأوراق نبات الكاربس *Conocarpus erectus* على الفطرين *Alternaria solani* و *Ulocladium botrytis* المعزولين من جذور نبات خيار القثاء *Cucumis melo* var. flexuosus المصاب بالتبقع وتعفن الجذور . وعند معاملة الفطر *A. solani* كانت أعلى نسبة تثبيط هي (100)% بالنسبة للمستخلص الايثانولي والميثانولي و(78.3 و 77.7 %) بالنسبة للمستخلصات المائية الباردة والحارة على التوالي عند التركيز (45) ملغم / مل . وفي حالة الفطر *U. botrytis* كانت أعلى نسبة تثبيط (100%) أيضاً بالنسبة للمستخلصات الايثانولية والميثانولية عند التركيزين 30 و45 ملغم / مل أما المستخلصات المائية الحارة والباردة وكانت نسبة التثبيط (88.8 و 86.1 %)على التوالي عند التركيز(45) ملغم / مل .

وأجري اختبار تأثير المستخلص النباتي أعلاه في أنبات بذور نبات خيار القثاء فوجد أن نسبة الإنبات انخفضت في تركيز (15) ملغم / مل من المستخلص المائي البارد والايثانولي والتركيز(30) ملغم / مل من المستخلص الميثانولي أما التراكيز الأخرى كانت مقاربة لنسبة الانبات في السيطرة .

The effect of leaves extract of (*Conocarpus erectus* L.) plant on *Ulocladium botrytis* and *Alternaria solani* fungi that isolated from

(*Cucumis melo* var. flexuosus) roots

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Abstract:-

The effect of cold and hot water , methanol and ethanol extracts of leaves of *Conocarpus erectus* plant on *Alternaria solani* and *Ulocladium botrytis* fungi isolated from *Cucumis melo* var. flexuosus roots were tested . When treatmented *Alternaria solani* the (100%) highest inhibition percentage of methanol and ethanol extracts and (78.3 , 77.7%) for cold and hot water extracts, respectively on (45) mg/ml . In the case of *Ulocladium botrytis* also the highest percentage (100%) inhibition ofmethanol and ethanol

extracts in (30 and 40) mg/ml , as hot and cold water extracts inhibition percentage above of (88.8 and 86.1%) respectively on (45) mg/ml. Tested effect plant extracts in germination of plant , It was found that the percentage of germination was decreased at the concentration (15) mg/ml of cold water and ethanol extracts and of (30) mg/ml of methanol extract ; The other extracts were approximate to germination percentage of control .

Introduction :-

Plants have formed the basis of natural pesticides that make excellence lead to new pesticides development (Arokiyaraj *et.al.* , 2008 ; Milinad *et.al*. , 2009) Many plants provided abundant resources of antimicrobial compounds used for centuries to inhibit microbial growth (Jun *et.al.* , 2006) for example , alkaloids , phenols , flavonoids , resins and tannins that are soluble in methanol and ethanol (Holetz *et.al*., 2002 ; Perez *et.al.*, 2003) . Bajwa *et.al*, (2003) Conducted a study to reduce the biomass of *Aspergillus niger* and *Phoma glomerata* using water extract for *Parthenium hysterphrus* and using the plant extract of *Eugenia aromatic* L inhibiting effectiveness *Fusarium* spp and *Alternaria* spp causing diseases spot and wilt in potato and tomato plants ; therefore , the aim of this study was to investigate the antifungal activity of *conocarpus erectus* (family: combretaceae) ; The plant was astringent and used to treat anemia ,diabetes , fever, gonorrhea, headache , bleeding, tumors and syphilis. The leaves were eaten or boil water and drunk to treat fever (Irvine, 1961 ; Duke and Wain, 1981) , for testing the antifungal activity for this plant selection plant pathogenic fungi *Alternaria solani* and *Ulocladium botrytis* isolation from spot and rot the roots of *Cucumis melo* var. flexuosus .

Collection of plant leaves :-

Fresh leaves of *Conocarpus erectus* were collected from the garden of the college of sciences , university of Thi-Qar . Washing with tap water and once with sterile water and dried at room temperature and pulverized into powder with an electric blender (Matawalli *et.al*., 2004)

Fungal species :-

Two fungal species were obtained from the science college of Basrah university isolated from *Cucumis melo* var. flexuosus

infected by spot and rot roots

Phytochemical analysis of extracts :-

This character was carried out by using standard procedure as described by Harborne (1984) the procedure was for alkaloids , glycosides , saponins , tannins , resins, phenolic compounds and steroids .

Preparation of extracts :-

1- Hot aqueous extract :-

The hot aqueous extract was prepared by extracting of 30 gm of plant powder with 300 ml of distilled water and putting in the water path for 30 min . Then, the mixed filtered and dried at a laboratory temperature . crude extract was collected and kept in a laboratory till uses (Suleiman , *et.al*. , 2008)

2- Cold aqueous , ethanol and methanol extracts :-

It was added (30) gm of plant powder to 300 ml from the solvent (aqueous , ethanol and methanol) and let it in shaker apparatus for (24) h . because each extract was filtered through Whitman filter paper No1 and concentrated by rotary evaporator at (40) C . The extract of plant was stored in the refrigerator at (4) C (Adekunle and Ikumapay , 2006 ; Wagat *et.al.* , 2008 ; Sama and Ajaiyeoba, 2006) .

The activity of extracts :-

The activity of the crude extracts were evaluated according to the reported procedures of Zacchino *et.al.* (1999) using agar dilution method 1gm extract was disolved in 5 ml of distill water to obtained the concentration (200) mg/ml (stock solution) and to prepare of concentration (15 , 30 , 45) mg/ml and added to sterill potato dextrose agar (18.5 , 17 , 15.5) ml and let for hardening and Inculcated by disks from fungal cultured (5 days old) control Petri dishes (added of distill water) . All the Petri dishes were incubated at (28) C . Then , the percentage of inhibition were calculated by using the following formula :-

$$I\% = \frac{Dc - dt}{Dc} \times 100$$

I = percentage of inhibition

Dc = Diameter of colony on control culture

Dt = Diameter of colony on treated culture

The effect of plant extracts on the germination of plant seeds :-

The peatmoss moistened with distill water and then putting in cloth bags to be steril in autoclave (121 C and pressure of 15 pounds/Ing for 30 minutes) Then, the dishes (15 cm diameter) were used and sterile peatmoss (1cm) thickness added , then distributed by subtracting plant seeds (10 seeds per dish) and 15 ml of plant extracts were added in the concentrations (15 , 30, 45) mg/ml and Repeater three times for each treatment . Comparative dishes at the same way were prepared used distill water instead of plant extract at the all dishes were incubated at a temperature (30) C for 7 days and, then the rate of germination percentages were calculated as in the following formulla :-

$$\text{Rate of germination} = \frac{\text{Number of seeds germination}}{\text{Number of total seeds}} \times 100$$

(Al-Haidery , 2007)

Statistical analysis :-

Tests were carried out by using design data that statistically analyzed by the complete randomness using variance analysis and estimation of Least significant difference (LSD) at the level of probability (0.05) after converting percentages to angular conversion values (Al- Rawi and Kalaf-Allah , 2000) .

The results :-

The test impact of processed extracts from the plant leaf powder ***Conocarpus erectus*** inhibition the growth of *U. botrytis* and *A. solani* , where the extracts contained alkaloids , saponins , tannins , phenplic compound , resins and terpins , then the effectiveness of plant extracts were tested in inhibiting the fungi above . It was found that cold water extraction of plant achieved an inhibition percentage to *A. solani* is (%78.3) when the highest concentration is (45) mg/ml and when the treatment of the same fungus by hot water extracts are not significantly different from cold extract , if inhibition percentage was achieved to (%77.7) in the same concentration . When *U. botrytis* was treated by water extracts , inhibition percentage was (%88.8) in using cold water extract and the treatment by hot water extract inhibition percentage was (%86.1) in (45) mg/ml and also there was no significant differences between transactions , through results using hot and cold water in inhibition fungi showed no significant differences at all concentrations , whereas the treatments by ethanol and methanol extracts reached percentages of inhibition to (%100) at (45) mg/ml for *A. solani* while it *U. botrytis* reached percentages of inhibition to (%100) at (30 and 45) mg/ml . The results obtained the extract activity of *Conocarpus erectus* (leaves) showed that the plant extract possess antifungal properties and could be effective pesticide . The different percentages of inhibition probably was due to the quantity of the phytochemical compounds were present in the extracts .

The tannin and phenolic compounds had antimicrobial activities, low concentration tannins could inhibit the growth of microorganisms and act as an antifungal agent at higher concentration by coagulating the protoplasm of the microorganisms (Onadapo and Owonubi , 1993) . The bioactive constituents of *Conocarpus erectus* might be glycosides , alkaloids , phenolic compounds and tannins since the presence of these listed phytochemicals in other plants have been reported by Barnabas and Nagarajan (1988) , Burapedjo and Bunchoo (1995) and implicated to inhibit cell wall formation in fungi leading to the death of the organism .

Table 1 :- the chemical tests

Compounds	Type of reagent	The guide	Result
Glycosides	Benedict reagent	Red precipitate or brown	+

Alkaloids	Marquis reagent	Cream precipitate	+
Phenolic compound	Iron chloride %1	Red color	+
Tannins	1- lead acetate 2- Iron chloride	- white recipitate - bluish green	+
Resins	Acidic water & ethanol	Turbidity	+
Saponins	Mercury chloride	white precipitate	+
Terpens & Steroids	Liberman –burchard	bluish green color	+

Table 2 :- The percentage for the effect of plant extracts on *A. solani*

Concentration mg / ml	Hot water	Cold water	Ethanol	Methanol
15	55.5b	72.2a	67.7c	64.4c
30	76.6a	75a	85.5b	72.2b
45	77.7a	78.3a	100a	100a
Control	0.0c	0.0b	0.0d	0.0d

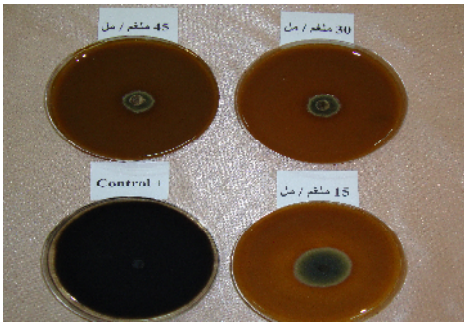
- Each value is the rate of three Repeater

- Percentages converter angul

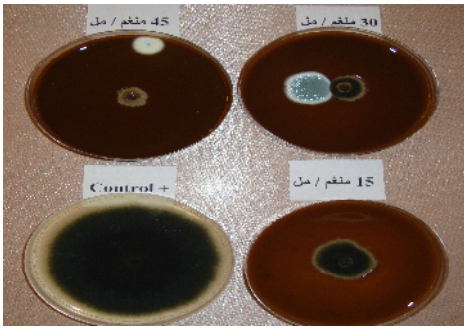
Table 3 :- The percentage for the effect of plant extracts on *U. botrytis*

Concentration mg / ml	Hot water	Cold water	Ethanol	Methanol
15	70b	76.1b	71.1b	b76.6
30	82.7a	82.7a	100a	100a
45	86.1a	88.8a	100a	100a
Control	0.0c	0.0c	0.0c	0.0c

- Each value is the rate of three Repeat



Picture (1) Effect of hot water extract from plant leaves on Ulocladium botrytis



Picture (2) Effect of cold water extract