

Antifungal activity of seven plant extracts against root rot fungi infected cantaloupe plant

Adel S. K. , Sabry I. M. , Ashraf M. M., Saeed Z. K

1- Envi University of Sadat City

2-Faculty of Agriculture al-manoufia University

ABSTRACT

The study dealt with the antifungal activity of some plant extracts against the fungi of mold rot of the roots that infect the roots of the cantaloupe plant, where the affected cantaloupe plant roots were collected from different places in Egypt, including (Nubaria _ Khattaba _ Medina Sadat _ Egypt's Desert Road, Desert Road kilo 76) and the *Fusarium Solani* fungi and fungi were isolated. Lithium - Solanese Resect Fungus, seven wild plants were collected, then plant extracts were made using an organic solvent (methanol) and the results of an anti-fungal activity experiment showed that all the plant extracts led to a significant inhibition of the growth of the tested fungi. Inhibition against *Fusarium solani* fungi 52.6 mm and against fungicide solani fecunda 22.2 mm and proven studies of Walter Keba minute the effect of plant extract of the taxa on the cellular composition of *Fusarium solani* fungi studies showed an increase in the thickness of the fungal cell wall. Chemical analysis of the extract of the tax collector using the HPLC device. The plant extract of the tax collector contains phenolic and flavonoids, which are important materials to inhibit the growth of fungi.

Key words: Antagonistic activity, *Trichoderma Spp.*, root rot pathogens,

ملخص

اتناولت الدراسة التأثير المضاد لبعض المستخلصات النباتية ضد فطريات اعفان الجذور التي تصيب جذور نبات الكنتالوب حيث تم تجميع جذور نبات الكنتالوب المصابه من اماكن مختلفه من مصر منها (النوباريه _ الخطاطبه _ مدينه السادات _ طريق مصر اسكندريه الصحراوي كيلو ٧٦) وتم عزل فطريات الفيوزاريوم سولاني_ وفطر البثيوم_ وفطر الريزكتونيا سولاني وقد تم تجميع سبع نباتات بريه ثم عمل مستخلصات نباتيه باستخدام مذيب عضوي (الميثانول) ونتائج تجربه النشاط المضاد للفطريات اتضح ان جميع المستخلصات النباتيه ادت لتثبيط معنوي لنمو الفطريات المختبره وكان مستخلص نبات العشار اكثر تثبيطا ضد فطر الفيوزاريوم سولاني ٥٢,٦ ملم وضد فطر ريزكتونيا سولاني ٢٢,٢ ملم واثبتت دراسات التر كيبا الدقيقه تأثير مستخلص نبات العشار على التركيب الخلوي لفطر الفيوزاريوم سولاني اوضحت دراسات زياده سمك جدار الخليه الفطريه. تشكلت السيتوبلازم. وجود اجسام دهنيه كبيره ادت الى تدمير لمحتويات الخليه الفطريه واثبتت دراسات التحليل الكيماوي لمستخلص نبات العشار باستخدام جهاز HPLC احتواء المستخلص النباتي للعشار على مواد فينولييه وفلافونيدات وهي مواد هامه لتثبيط نمو الفطريات وبالتالي يعتبر مستخلص العشار بديل للكيماويات في مقاومه فطريات اعفان الجذور مثل فيوزاريوم سولاني وريزكتونيا سولاني التي تصيب جذور الكنتالوب.

كلمات دالة: root rot pathogens- *Trichoderma Spp*- Antagonistic activity:

INTRODUCTION

Due to plant diseases every year nearly 10-20% of the total world food production was decreased and leads to loss of billions of dollars. Chemical control is offer nonspecific in its effect, may can cause killing beneficial organisms and it may have undesirable health and environmental pollution risk. Intensified used of fungicides has resulted in accumulation of toxic compound potentially hazardous to human and environment and also in the build up of resistance of the pathogens. In order to tackle

these national and global problems, effective alternatives to chemical control are being employed. Biological control is nature friendly approach that uses specific micro-organisms, which interfere with plant protection. Biological control by an antagonism is a potential, no chemical and ecofriendly approach for managing plant diseases (Bailey *et al.*, 2004).

Fusarium oxysporum is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms in many crop plants (Cotxarrera, *et al.*, 2002). The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield (Nemec *et al.*, 1996). *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *Lycopersici* (FOL) is the major limiting factor in the production of tomato. The disease causes great losses, especially on the susceptible varieties of tomato especially when soil and air temperature are rather high during the warm season (Agrios, 1997, Mandal *et al.*, 2009). The interaction between *F. oxysporum* and *F. solani* causes a root-rot disease complex that severely damages this important crop (Klotz, 1973).

Biological control of plant diseases is considered as one of the viable alternative methods to manage plant diseases (Barakat & Al-Masri, 2005; Pal & Gardener, 2006). The application of biological controls using antagonistic microorganisms has proved to be successful for controlling various plant diseases in many countries (Janisiewicz, 2000). Most of these studies were on the control of root and soil-borne plant pathogens and, to a lesser extent of foliar pathogens (Whipps & Lumsden, 2001).

The antifungal activity of 36 plant aqueous extracts have been evaluated against *F.oxysporuin*, *R.solani* and *B.cinerea* in *vitro* testes and under greenhouse condition on watermelon and tomato plants (Elkaffash and Al-Menoufi,2003). They found that clove extract completely inhibited the mycelial growth of the three tested fungi whereas garlic extract suppressed the mycelial growth of *F.oxysporinna* and *B.cinerea* by 100 % and *R.solani* by 88 %. In the same year, Dawood *et al.*, (2003) evaluated 11 aqueous plant extracts for their antifungal activity against *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Sclerotinia sativa*, *Alternaria alternata*, *Aspergillus flavus*, *Botrytis cinerea*, *Sclerotium rolfsii* and *S.batiticola*. They stated that Spearmint (*Mentha viridis*) extract had no effect on mycelial growth of any of the tested fungi.

The objective of this study was to evaluate the potential of the plant extracts of some wild plants in controlling the root rot fungi *Rhizcoctonia solani* and *Fusarium solani* which infected Cantaloupe plant under in *vitro* condition .

1. Materials & Methods

1.1. PHYTOPATHOLOGICAL STUDIES

1.1.1. Sampling

Naturally diseased Cantaloupe root showing wilting symptoms, Cantaloupe plants showing wilting and root rots were collected from different districts of Egypt namely Sadat city, Noberia, el kataba, and Alex. Egypt road K. 76. Collected samples were transferred to laboratory in plastic bags to be used in pathogen isolation.

1.1.2. Phytopathogenic Fungi

Isolation, identification and pathogenicity test were carried out for, *Fusarium solani* and *Rhizoctonia solani*. The isolates were grown on potato – dextrose agar (PDA) medium (see-appendix) in petri dishes, then transferred to PDA slants and kept in refrigerator at 4°C as a stock cultures.

1.1.3. Isolation, purification and identification of *Fusarium solai*

Diseased cantaloupe plants showing different degrees of wilt disease were collected from different regions of, (Egypt Sadat city, Noberia, El kataba, and Alex. Egypt road K. 76).

The affected roots were washed thoroughly with running tap water and cut into small portions before immersing in Sodium hypochlorite (5% chlorin) for one minute. The surface was washed with distilled water and finally dried between two sterilized filter papers. Then they were directly placed into Petri dishes containing potato dextrose agar medium (PDA) and incubated at 28°C for 3-5 days. Examination was carried out when fungal growth appeared out from the incubated materials. All the isolated fungi were purified using single spore or the hyphal tip techniques suggested by Dhingra and Sinclair (1985). The purified fungi were identified according to their morphological features according to Booth (1985). Stock cultures were maintained on PDA slants and kept in a refrigerator at 5-10°C and were sub-cultured on fresh medium every 6-8 weeks.

1.1.4. Isolation, purification and identification of *Rhizoctonia solani*

The collected diseased cantaloupe roots showing damping-off disease were collected from different regions of washed in running tap water followed by sterile water. Using sterilized scalpel, roots were cut into small pieces (1-2 cm²). The pieces were then transferred into 0.1% hypochlorite solution (disinfectant solution) for 3 minutes for surface sterilization. Surface sterilized pieces were then washed several times with sterilized water to wash out the remaining disinfectant solution. The pieces were then dried on sterilized filter papers. Using sterilized forceps, dried pieces were then transferred into Petri dishes containing potato dextrose agar medium (PDA) supplemented with antibacterial agent (L-chloramphenicol 5mg/L and streptomycin sulphate 5mg/L). The dishes were then incubated at 28°C. and checked for fungal growth two days later. The resulting isolates were purified using mycelium fragment. (Shabana, 1987). Pure cultures of the isolated fungi were identified according to the

cultural properties, morphological and microscopical characteristics described by Domsch *et al.*, (1980)

1.2. COLLECTION OF WILD PLANTS

Seven wild plants (Table 1) were collected from canal banks, fields and markets of various localities in El menofoia governorate and were taxonomically identified by Prof. Dr. Mohamed Fathy Azazy Surveys of Natural Resources Dept , Environmental Studies and Research Institute, University of Sadat City according to Tackholm (1974) and adopted according to Boulos (1999-2005).

Table (1): The collected wild plants

No	Scientific name	Family	Arabic name	part used
1	<i>Azadicachta indica</i> Adrjuss	Meliaceae	النيم	Leaves
2	<i>Calotropis procera</i> (Aiton)W.T. Aiton	Asclepiadaceae	العشار	Leaves
3	<i>Cymbopogon proximus</i> (hochst) staps.	Poaceae	الحلغابير	Leaves
4	<i>Cyperus rotundus</i> L.	Cyperaceae	السعد	Leaves
5	<i>Eucalyptus rostrata</i> Schlecht	Myrtaceae	الكافور	Leaves
6	<i>Nerium oleander</i> L.	Apocynaceae	الدفلة	Leaves
7	<i>Pluchea dioscoridis</i> L.	Asteraceae	البرنوف	Leaves

1.3. PREPARATION OF PLANT EXTRACTS

Plant materials were dried in the shade at room temperature, ground using electrical mill into fine powder and extracted by soaking in methanol at the rate of 1:3 (w/v) for 48 hours.

The extracts were filtered through cheese cloth under a strong hand pressure and the solvent was dried under vaccum at 60-65C° using a rotary evaporator. The extracted residue was dissolved in dimethyl sulfoxide (1mg /ml). The crude extracts were preserved under refrigeration until use (Dawood *et al.*,2003).

1.4. Screening of different plant extracts for antifungal activities against the selected phytopathogenic fungi

Seven plant extracts (Table 1) were examined for their inhibitory activities against some selected phytopathogenic fungi by using agar diffusion technique (Deans and Ritchie,1987).

Dry potato dextrose agar plates were inoculated by spreading spore suspensions of constant fungal inoculum. In case of *R.solani*, discs were putted on the surface of plates because this fungus grow as mycelia or sclerotia. Wells were made in the inoculated potato dextrose agar plates for plant extracts and essential oils inoculation

(200 μ). All plates were incubated at 26 \pm 2 $^{\circ}$ C for 72 hours. The diameter of inhibition zones were determined for all tested fungi except *R.solani*, the radius of inhibition zones were measured.

1.5. Phytochemical analysis by HPLC:

The chemical composition of *Sargssum species* extract were performed in central labs unit, Faculty of Agriculture, Cairo University, Gizza, Egypt for detected phenolic, flavonoids and other active compounds.

1.6. Ultrastructure studies (T.E.M):

The method described here is an example for fixation, contrastation and embedding only. For optimal results especially the fixation needs to be adapted to the investigated tissue. It is of major importance that the fixing agent is able to completely penetrate the specimen as soon as possible. For this reason only very small pieces of tissue (~ 1 mm³) should be fixed if no perfusion fixation (fixative is directly infused into a larger vessel of a deeply anaesthized animal) should be possible. The preparation should be quick enough, i.e. 5 minutes after tissue does no longer receive oxygen, and it began to show first signs of degeneration of ultrastructures. Perfusion or immersion fixation of the tissue using a modified Karnovsky (1965) solution: 2.5 % buffered glutaraldehyde + 2 % paraformaldehyde in 0.1 M sodium phosphate buffer pH 7.4, leave tissue overnight at 4 $^{\circ}$ C, wash 3 x 15 minutes (min.) in 0.1 M sodium phosphate buffer + 0.1 M Sucrose, postfix 90 min. in 2 % sodium phosphate buffered osmium tetroxide pH 7.4, wash 3 x 15 min in 0.1 M sodium p, after drying for ~15 min sections may be investigated in a transmission electron microscope. Ultrathin sections were observed at 160 kV using a JEOL JEM - 2100 at Electron Microscopy Unit, Mansoura University, Egypt.

1.7. Statistical analysis:

All values represent the mean of triplicate determinations. Data were statistically analyzed by using one way analysis of variance (ANOVA) according to SPSS (1999). The least significant difference is abbreviated as LSD and measured at $P \leq 0.05$.

2. RESULT

2.1. Psychopathological Studies

2.1.1. Isolation, purification and identification of the causative organism of wilt disease (*Fusarium solani*)

The causative organism of wilt disease was isolated from diseased cantaloupe roots collected from different regions of Egypt namely Sadat city, Noberia, el kataba, and Alex. Egypt road K. 76. These isolates were identified as *Fusarium solani* (Fig.1) according to morphological and microscopic features (mycelial development and spore formation) according to Booth (1985). The identification was confirmed by Mycology Center, Assuit University as (*Fusarium solani* 9704 AUMC).



(1): Conidia of *Fusarium solani* (400x) light microscope

2.1.2. Isolation, purification and identification of the causative organism of damping-off disease on bean (*Rhizoctonia solani*)

The causative organism of damping off disease was isolated from diseased cantaloupe roots which collected from different regions of Egypt namely Sadat city, Noberia, el kataba, and Alex. Egypt road K. 76. The isolates obtained were purified using mycelium fragment and these isolates were identified as *Rhizoctonia solani* (Fig.4) according to morphological and microscopical characteristics by Domsch *et al.*, (1980). The identification was confirmed by Mycology Center, Assuit University as (*Rhizoctonia solani* 6590 AUMC).



Fig (2): Mycelium of *R.solani* (400 x) light microscope

2.2. Screening of plant extracts for antifungal activities against *Fusarium solani*

Seven plant extracts were screened for their antifungal activities against *Fusarium solani*. They are arranged in Table (2) and Fig. (3) according their antifungal actitives. *Calotropis procera* was the most active plant extract against *Fusarium solani* followed by *Azadicachta indica* Adrjuss, *Cymbopogon proximus* (hochst) staps, *Cyperus rotundus* L., *Eucalyptus rostrata* Schlecht, *Nerium oleander* L. and *Pluchea dioscoridis* L.

Table (2): Screening of plant extracts for antifungal activities against *Fusarium solani*

NO	The plant extract	Diameter of inhibition zone (mm)●
	Control (DMSO)	18.0
1	<i>Calotropis procera</i> (Aiton) W.T. Aiton	52.6*
2	<i>Azadicachta indica</i> Adrjuss	48.3*
3	<i>Cymbopogon proximus</i> (hochst) staps.	41.5*
4	<i>Cyperus rotundus</i> L.	38.5*
5	<i>Eucalyptus rostrata</i> Schlecht	37.5*
6	<i>Nerium oleander</i> L.	37.5*
7	<i>Pluchea dioscoridis</i> L.	37.3*

LSD = 2.1

●The recorded valued are the mean values of 3 replicates

* Significant

Non significant

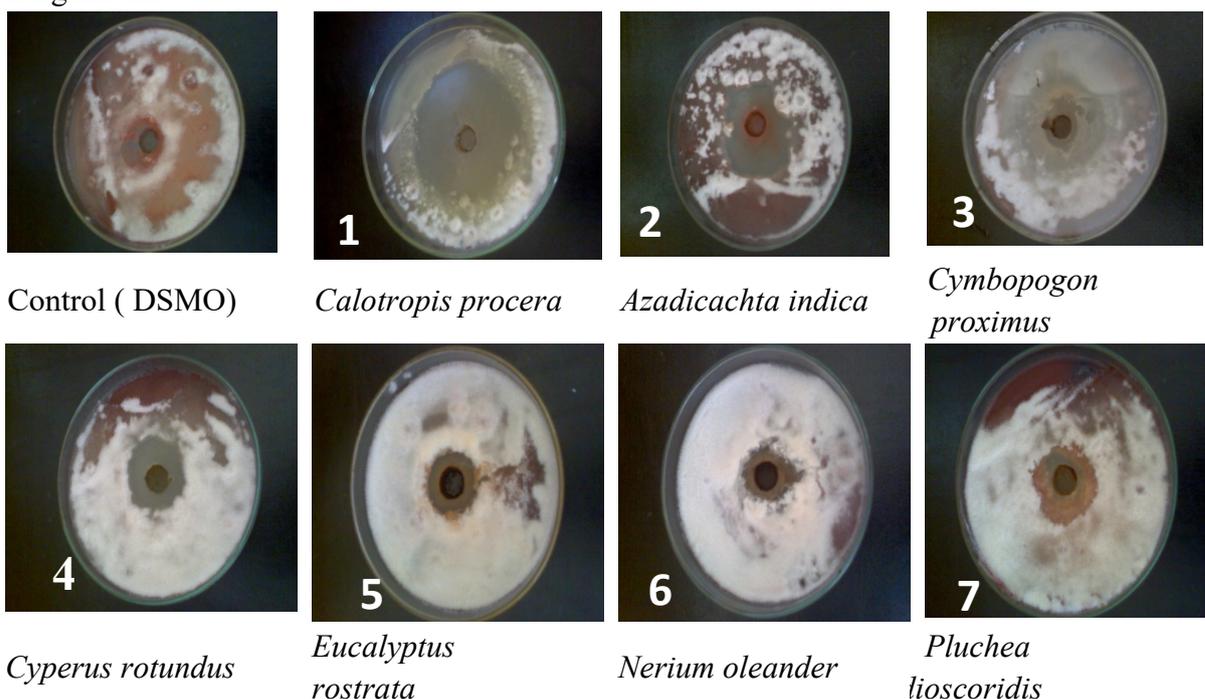


Fig (3): Screening of plant extracts for antifungal activities against *Fusarium solani*

2.3. Screening of plant extracts for antifungal activities against *Rhizoctonia solani*

Data in Table (3) revealed that seven plant extracts were screened for their inhibitory activities against *Rhizoctonia solani*. They are arranged in Table 3) and Fig.(4) according to their inhibitory activities. The plant extract of *Calotropis procera* was the most active against *R.solani* followed by *Eucalyptus rostrata* Schlecht,

Azadicachta indica Adrjuss, *Cyperus rotundus* L., *Nerium oleander* L., *Cymbopogon proximus* (hochst) staps. and *Pluchea dioscoridis* L. respectively.

Table (3): Screening of plant extracts for antifungal activities against *Rhizoctonia solani*

No	Plant extract	Radius of inhibition zone (mm)*
	Control (DMSO)	4.0
1	<i>Calotropis procera</i> (Aiton) W.T. Aiton	22.2*
2	<i>Eucalyptus rostrata</i> Schlecht	20.1*
3	<i>Azadicachta indica</i> Adrjuss	19.8*
4	<i>Cyperus rotundus</i> L.	14.8*
5	<i>Nerium oleander</i> L.	14. 6*
6	<i>Cymbopogon proximus</i> (hochst) staps.	11.7*
7	<i>Pluchea dioscoridis</i> L.	11.5*

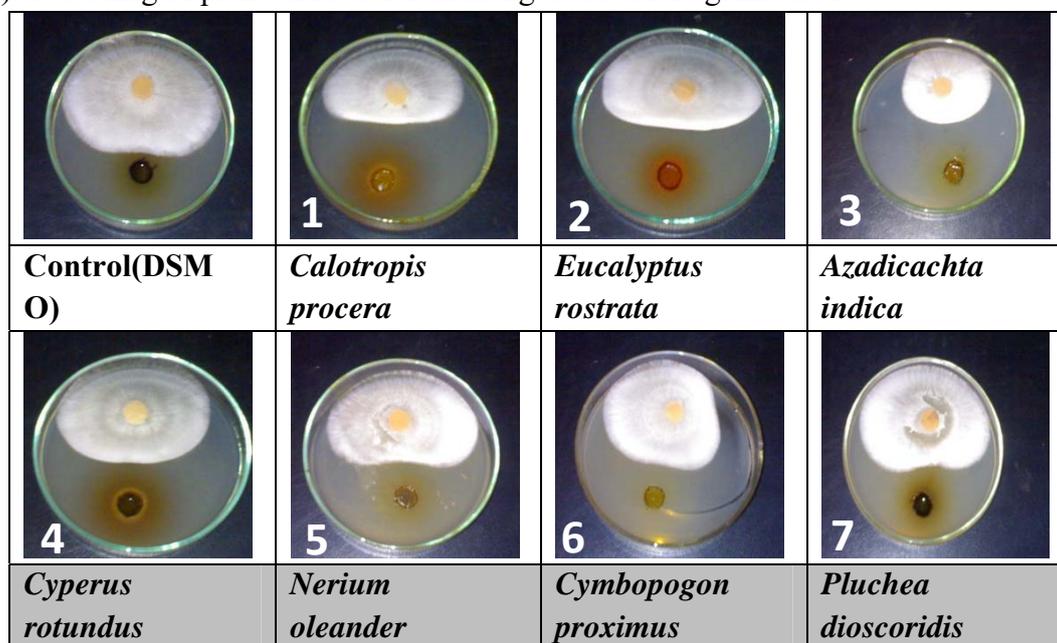
LSD = 0.1

●The recorded valued are the mean values of 3 replicates

* Significant

Non significant

Fig (4): Screening of plant extracts for antifungal activities against *Rhizoctonia solani*



2.4. Transmission electron micrographs showing the effect of plant extract of *calotropis prcera* on *Fusarium solani*.

A: *fusarium solani* hypha grown on medium lacking plant extract of *calotropis prcera*. A cell of *fusarium solani* surrounded by a thin wall (W) and the cytoplasm (Cy)

contains numerous organelles such as mitochondria (M). Note an electron-dense plasmalemma (arrow). Scale bar = 0.5 μ m (cited from Baka *et al.*, 2002).

B and C: *Fusarium solani* hypha grown on medium containing plant extract of *calotropis prcera* at concentration of 0.3%. *Fusarium solani* cell show a thickening wall (w), disintegration of cytoplasm (Cy). Note large lipid bodies (L), vacuoles (v) and not recognized plasma membrane. Scale bar=0.5 μ m.

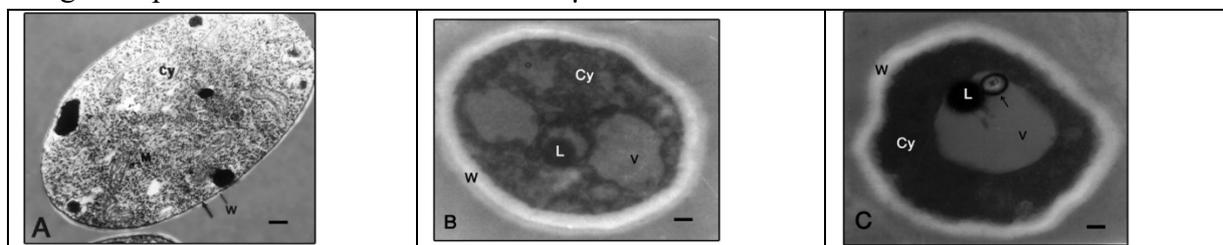


Fig. (5) Transmission electron micrographs showing the effect of plant extract of *calotropis prcera* on *Fusarium solani*.

2.5. Phytochemical analysis by HPLC:

The results in Table (4) showed Phytochemical analysis of *calotropis prcera* extract showed presence of phenolic compound and flavonoids which responsible for antifungal activities.

Table (4): Phytochemical analysis by HPLC:

Compound	conc. (ug/g)
Gallic acid	0.000
Protocatechuic acid	15.919
<i>p</i> -hydroxybenzoic acid	29.767
Gentisic acid	0.000
Catechin	134.522
Chlorogenic acid	31.668
Caffeic acid	0.000
Syringic acid	8.148
Vanillic acid	5.080
Ferulic acid	200.160
Sinapic acid	115.025
<i>p</i> -coumaric acid	0.000
Rutin	183.061
Apigenin-7-glucoside	0.000
Rosmarinic acid	0.000
Cinnamic acid	5.673
Quercetin	0.000
Kaempferol	106.245
Chrysin	0.000

Discu Thus, our study was planned to replace the undesirable and unsafe chemical control by another effective, inexpensive and safe options for control of some root rot diseases in Cantaloupe Several strategies were used for biological control of

plant pathogens by using plant extracts (Assadi and Behroozine,1987 Natural plant extracts may provide an alternative to chemicals for controlling plant disease (Farag *et al.*, 1989).

The screening of antifungal activity of seven plant extracts against phytopathogenic fungi, *F solani* , *R.solani*, showed that plant extracts have antifungal activities against the tested phytopathogenic fungi. Among all plant extracts tested the plant extract of *calotropis procaera* proved to be the highest most effective growth inhibitor for all the tested phytopathogenic fungi. Also the results revealed that the other plant extracts have high antifungal activities against the tested phytopathogenic fungi. These results were in agreement with that of Wilson *et al.*,(1997) who tested extracts from 345 plants for their antifungal activities against *Botrytis cinerea*. They found that among the 345 plant extracts tested, 13 showed high levels of antifungal activities, with species of *Allium* predominating.

Also, Carcia and Lawas (1990) studied the antifungal activity of the crude water extract of 127 plant species against *R.solani* and they found that garlic extract (*Allium sativum* was the most effective one Mahmoud *et al.*,(2004), reported that, the plant extracts of *Ipomoea carnea*, *Eucalyptus citriodora*, *Cuminum cyminum*, *Allium sativum* and *Hyocypus muticus* have antifungal activities against *Botrytis fabae* and showed that the plant extracts of *Eucalyptus citriodora* and *Ipomoea carnea* have high inhibition zones against *B.fabae* also have high ability to decrease percentage of spore germination of *B.fabae* .

The antifungal activities of plant extracts may be attributed to the plant contents of secondary metabolites (e.g. phenolic, alkaloids, flavonoids, and terpenoids) that could adversely influence pathogen growth and development (Cowan,1999). Some plants impact on the growth and or development of others by releasing various chemical compounds called allelopathy (Jadhav *et al.*,1997).

3. Also, Ranjan *et al.*, (1991) studied the activity of 15 plant extracts on aflatoxin production and growth of *Asp. flavus*. They observed that extract of *Artemisia indica* and other 5 plants inhibited the production of aflatoxins B1 and B2 by more than 50%. Moreover, Ismail (1998) tested the antifungal activity of aqueous extracts of 7 plants against *R.solani* and *F.oxysporum*. *Artemisia Judaica* had a moderate inhibition effect on the linear growth of the tested fungi. In the present work, the transmission electron microscope (TEM) examination of sections from *f solani* grown in the presence of 0.3% plant extract of *calotrops procera* showed a pronounced cellular disorganization of the fungus. These changes include distorted cell walls, no discernible organelles, increase of lipid bodies and vacuoles and disintegration of the cytoplasm. These results were in agreement with that of Bianchi *et al.*, (1997) who reported that transmission and scanning electron microscopy revealed cytomorphological alterations of the hyphae of *Collectotrichum lindemuthianum*, *Rhizoctontia solani* and *Fusarium solani* treated with garlic extract Nakamura *et al.*, (2004) reported that the analysis of the ultrastructure of yeast cells treated with the essential oil of *Ocimum gratissum* revealed changes in the cell wall and in the morphology of some subcellular organelles and also reported that the essential oil of *Ocimum gratissum* has antifungal activities and eugenol is the main constituent of this essential oil and is thought to be mainly responsible for the antifungal effect.
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