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Effectiveness of *Conocarpus lancifolius* Extract against Insects and Pathogenic Fungi

Ismail Ibrahim Alyaseri, Fatimeh Qassem Hamdan, Ghasan Mahdi Dagher and Noreen Abdulzahra Hasan

Department of Plant Protection, Faculty of Agriculture, University of Misan, Iraq
E-mail: ismailalyaseri@uomisan.edu.iq

Abstract: The study was carried out in the Faculty of Agriculture- University of Misan, to investigate the ability of the *Conocarpus lancifolius* extract to control some fungi and insects. Experiments were carried out in the laboratory (using the ethyl alcohol extract in concentrations 0.5, 1 and 1.5%) and the plastic house(using the butanol extract in the concentrations 1.5, 3 and 4.5%) to determine the ability of the extract to inhibit the growth of the fungi *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, and laboratory study (using the butanol extract in the concentrations 1.5, 3 and 4.5%) to determine the efficiency of the extract to control the insects *Myzus persicae* and *Aphis nerii*. The effect of the conocarpus extract was high significant with 1.5% concentration on the three fungi in the laboratory. The results of the plastic house indicated that the effect of the extract was relatively better when used on eggplant. *Fusarium oxysporum* was unable to infect both eggplant and tomato when the extract was used in the three concentrations after 10 days of addition of fungi. *Rhizoctonia solani* was unable to infect both eggplant and tomato when the extract was used in the three concentrations when the fungi were added with extract together. The results showed that the extract of conocarpus was highly significant effect on the insects *M. persicae* and *A. nerii*, where the percentage of killing was increased with the increasing of concentrations, they were of 78% and 79% for the concentration (4.5%) on *M. persicae* for tomato and eggplant respectively, and about 99% to the concentration (4.5%) after 4 days on *A. nerii*.

Keywords: *Conocarpus lancifolius, Fusarium oxysporum, F. solani, Rhizoctonia solani, Myzus persicae, Aphis neri*

In spite of the effectiveness of chemical pesticides in the control of agricultural pests, there are many problems associated with their use, including the impact on the components of the environment in general, so many of the researches and studies focused on the use of safe alternatives to the environment, including plant extracts, and there are many plants that contain materials Effective and toxic substances have clear effects on agricultural pests including insects, mites, fungi and others.

Studies have shown that the environmental conditions in Iraq are suitable for the cultivation of the conocarpus, Abid-Ali (2013) noted the ability of conocarpus plant to grow in the different Iraqi soil. When studying the resistance of *Conocarpus lancifolius* plants to four levels of salinity and three levels of water abundance. Mehwish Naseer et al (2017) studied the response of *Conocarpus lancifolius* to the process of photosynthesis under saline stress. Plant gave a good growth in the salinity level up to 300 millimose areas and some plant extracts showed harmful or anti-feeding effects as well as varying mortality rates on the red flour beetle *Tribolium castaneum* (Abu Kalish et al 2018). Ibrahim (2008) found that the extracts of some plants had a repellent effect of the potato tuber moth *Phthorimaea operculella*. Some extracts, vegetable oils and inert powders showed high efficacy as repellents for cowpea beetle *Callosobruchus maculates* (Ibrahim et al 2009), while Eilan (2014) explained that clove powder was highly effective in killing the same insect. Gamal (2008) reported that plant extracts of sweet almond, chamomile, sesame and black pepper killed all larval stages of the *Tribolium confusum*. Ghani (2014) noted that the extract of cesaban had a strong effect in the destruction of the larvae of the capillary beetle (Khabra) *Togoderma granarium*. The effect of some essential oils of mustard, camphor and peppermint on the vitality of the nymphs of *Ephedia kuehniella* (Alalan et al 2017), the results showed that the ethanolic extract of the flower blossoms of the carnation plant had an effect on the adults of the *Tribolium castaneum*. The lichen *Ebernia prunastri* extract showed a significant inhibitory effect in the growth of fungal filaments and spores production of *Fusarium oxysporum* and *F. solani* (Ali et al 2017). It was found that extracts from orange peel, lemon, basil and neem plant, either separately or mixed with each other, inhibited the growth of some fungi in the laboratory (Kashkiri et al 2006). In a laboratory study, Jassim (2017) pointed to the high effectiveness of the conocarpus extract in inhibiting the growth of *Alternaria alternata*, which causes the spots on the date palm leaves, the extract recorded the highest percentage in reducing the severity of the disease.

MATERIAL AND METHODS

Preparation of the extract: Leaves of conocarpus were

collected from the gardens of the Faculty of Agriculture, University of Misan, were used after washing and drying. They weighed 100 g and placed in an electric mixer, with the addition of 500 ml of ethyl alcohol (96%) then mixing and crushing for 10 minutes, filtration was done in a glass flask through a piece of gauze wrapped in glass funnel. Drain the extract by placing it in a 1000 ml glass beaker on the heater mantle at 45-50°C for 24 hours.

Preparation of the cultivation medium: A synthetic agar media (PDA) was used to prepare the medium for fungi cultivation, by dissolving 40 grams of the medium in one liter of water and mixed, then distributed in glass flasks (250 ml) well closed and placed in the autoclave with temperature at 121°C and pressure 15 pounds / inch^2 for 20 minutes.

Preparation of concentrations of the extract: Three concentrates of the conocarpus extract were prepared (0.5, 1 and 1.5%), by adding the extract to the flasks containing PDA (250 ml) after cooling and then distributed in 9 cm diameter Petri dishes. The fungicide Revus Top 500 SC (Difenconazole 250mg L^{-1} + Mandipropamid 250mg L^{-1}) was used with concentration 1ml L^{-1} by adding it to the PDA in the same way (for comparison).

Cultivation of fungi: The experiment carried out with three species of fungi, *Fusarium oxysporum* and *Fusarium solani* (obtained from the Faculty of Agriculture University of Basrah) and *Rhizoctonia solani* (isolated from the eggplant in the laboratories of the Faculty of Agriculture University of Misan). Parts of these fungi of a 2-week-old with a diameter of 0.5 cm taking with crock borer and placed on the PDA medium in the dishes prepared in the previous step with three replicates for each fungus and each concentration. Three dishes cultured with fungi were left without adding the extract (control).

E-Fungal growth account: The growth of fungi were measured in the dishes after 24 hours of treatment by measuring the diameter of the colony in centimeters, and continued recording of all treatments daily until the growth rate in the control treatment reach to 9 cm for each fungus mentioned in the previous paragraph.

Treatments: Treatments were included are given in Table:

G- Inhibition Rate: The percentage of inhibition was calculated based on the Abott equation (Awad Sha'ban and Nizar Mustafa 1993).

Percentage of inhibition = (Radiation growth rate in control - Radiation growth rate of treatment)/ Radiation growth rate in control × 100

Effect of conocarpus extract on fungi in plastic house

Cultivation plants: Tomato and eggplants obtained from the nursery of the Directorate of Agriculture-Misan (about 30 days old) placed in plastic pots capacity of 1 kg filled with soil added to the peat mouse by 10% , the soil was sterilized by the autoclave at a temperature of 121°C and pressure of 15 pounds inches^{-2} for 25 minutes.

Preparation of the extract: The extract was prepared with the same method as described in (1 - A) above but the solvent used was n- Butanol 99.5% instead of ethyl alcohol.

Fungi used: The same fungi used in the laboratory experiment: *Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani* (illustrated in 1-D above) were used in this experiment.

Treatments included the use of three concentrations of the extract (1.5, 3 and 4.5%), added to the ground soil in two methods, first was the addition of extract after ten days of the addition of fungi to the soil, and second was the addition of extract with the addition of fungi to the soil (in the same time) and by three replicates per treatment (three pots). The treatments are given in Table 1.

Second method: Same treatments (T1-18) were included with the addition of fungi and extract together (in the same time). The control treatments were the same as those specified in the first method.

Observation: The plants were left for 30 days after the treatment. Observations were recorded, the number of dead plants was calculated for each treatment and samples were taken to the laboratory for isolating fungi in the laboratory to confirm the fungal infection.

Effect of the conocarpus extract on some insects in the laboratory

Effect of the extract on *Myzus persicae* (Sulzer 1776)-

Preparation of the extract: The extract was prepared in the same method as mentioned in (1-A above) and used with three concentrations of 1.5, 3 and 4.5%. Tomatoes and eggplant leaves were dipping in Petri dish containing 20 ml of extract for each concentration. The leaves of tomato and eggplants planted in the plastic house were selected and placed in Petri dishes containing medical gauze plus 10 ml of sterile water after being immersed in the concentrations mentioned, with a wet piece of medical cotton on the end of leave vine. *M. persicae* insects collected from canola plants planted in the field of the Faculty of Agriculture, by 10 insects per dish, three replicates per treatment and left three replicates of each of the tomatoes and eggplant without extract and put the same number of insects for comparison(control). The number of live insects was recorded after 24, 48 and 72 hours after treatment. The percentage of killing was calculated based on the Henderson-Tilton equation (Henderson and Tilton 1955).

Effect of the extract on *Aphis nerii* (Boyer de Franscolombe 1841)

A-Preparation of the extract: This was as in above

experiment. The terminal buds of the oleander plants were cutting, placed in glass bottles containing sterile water and placed on each plant 25 insects of *Aphis nerii*, the plants sprayed with a small sprayer,containing 100 ml of extract in the three concentrations diluted with distilled water by three replicates per concentration. Three plants containing the same number of insects were left for comparison (control). The number of live insects was recorded after 24, 48, 72 and 96 hours, and the percentage of killing was calculated based on the Henderson-Tilton equation (Henderson and Tilton 1955).

Statistical analysis: The results were analyzed according to the complete randomized design (CRD) for all of the above experiments and the rates were compared based on the least significant difference (LSD) (Al-Rawi.,Khasha Mahmoud and Abdul Aziz Mohammed Khalaf 2000).

RESULTS AND DISCUSSION

Effect of conocarpus extract on fungi in vitro: The *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani* have diifernt period to complete the growth, where it was 6 days for *Fusarium oxysporum*, 12 days for *Fusarium solani*, and 15 days for *Rhizoctonia solani* (Table 1). The effect of fungicide indicate that three fungi, *Fusarium solani* was able to grow a small area after the second day of treatment (0.17 cm), the growth diameter was 2 cm after 12 days of treatment, while there was a slight growth of *Fusarium oxysporum* (0.3 cm) from the fourth day until the seventh day after the treatment. The fungicide inhibited the growth of the *Rhizoctonia solani* until 11days after the treatment, it grown with a small area of diameter (0.5 cm) until today (15) after treatment. The conocarpus extract (1.5%) inhibit the growth of the three fungi, the growth diameter was 3.17 cm of *Fusarium oxysporum*, 3 cm of *Fusarium solani* and 2 cm of *Rhizoctonia solani*. The effect of conocarpus extract was highly significant at 1.5% concentration on the three fungi.

The extract of the conocarpus had a significant effect on inhibiting the growth of fungi in the laboratory. The percentage of inhibition were 77.7, 66.6 and 64.7 for *Rhizoctonia solani*, *Fusarium solani*, and *Fusarium oxysporum* respectively when the growth of the control treatment reach to the edge of the dish (Table 1). Saad Toqeer et al (2014) observed that the methanol extract of the *Conocarpus lancifolios* was effective against four species of bacteria and the yeasts *Saccharomyces cerevisae* . The water extract of *Conocarpus lancifolius* showed an effect on *Meloidogyne incognita* on eggplant (Hussain et al 2016). The

Table 1. Effect of conocarpus leave extract on the growth of fungi *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Rhizoctonia solani* (RS) in the laboratory

Treatments**	Growth diameter (centimeter) in PDA after the period/days*														
	1*			2			3			4			5		
	FO	FS	RS	FO	FS	RS	FO	FS	RS	FO	FS	RS	FO	FS	RS
T1 (0.5%)	1.83	0.83	0.7	2.7	2.3	4.3	4.3	2.83	2.7	5.3	3.83	3.5	6.3	4.83	4.7
T2 (1%)	1.5	1.3	0.5	2.17	2.08	2.17	2.7	2.83	2	3.17	2.7	2.3	3.83	3.3	3.3
T3 (1.5%)	1.17	0.5	0.3	2	1.5	1.83	2.5	1.83	1.83	3	2.5	2.17	3.17	2.7	2
T4 (The fungicide)	0.0	0.0	0.0	0.0	0.17	0.0	0.0	0.17	0.0	0.3	0.5	0.0	0.3	0.83	0.0
T5 (Control)	3	0.5	0.7	4.7	1.83	2	5.83	2.3	2.5	7	3.3	3.3	8.5	4.3	4.17
LSD	0.33	0.78	0.4	0.4	1.19	0.58	0.81	1.13	0.67	0.9	1.15	0.8	0.85	1.43	1.4

Treatments	Growth diameter (centimeter) in PDA after the period/days*													
	6			7		8		9		11		12		15
	FO	FS	RS	FS	RS	FS	RS	FS	RS	FS	RS	FS	RS	RS
T1 (0.5%)	7.17	5.5	6.3	6.5	6.83	7	6.83	7.3	7.7	8.5	8.17	9	8.5	8.83
T2 (1%)	4.7	4.17	4.3	4.83	4.3	5.5	4.83	5.7	5.17	7.83	6.3	8.83	7	8
T3 (1.5%)	3.17	2.83	2	3	2	3	2	3.17	2	3.3	2	3	2	2
T4 (The fungicide)	0.3	1	0.0	1	0.0	1.3	0.0	1.5	0.0	2	0.5	2	0.5	0.5
T5 (Control)	9	5.3	4.7	6.3	6	6.83	6	7.3	7.17	8.5	7.7	9	8.3	9
LSD	0.74	1.4	0.68	1.38	1.76	0.96	1.75	0.93	1.4	0.78	0.9	0.62	0.47	0.23

T1,T2 and T3 are the concentrations of conocarpus extract , T4 was the fungicide Rovus Top 500SC and T5 was the control(without any addition)

effectiveness of plant extract may be due to the fact that it contains effective substances against microorganisms. Using thin layer chromatography (TLC), large numbers of alkaloids, phenols and serbtonin were separated from *Conocarpus lancifolius* (Touqeer et al 2015). Studies conducted by Malik Saadullah et al (2014) showed the presence of clocosides, tannins, terbenoids and sapones.

Effect of conocarpus extract on fungi in plastic house: The three fungi were unable to infect the eggplant plant when treated with the extract of the conocarpus (10 days after the addition of fungi) in the three concentrations, except one case of infection at the concentration of 1.5% to *Rhizoctonia solani*, and there was no infection with the fungicide for all treatments (Table 2). On the tomato plants, there was one infection with *Fusarium solani* at 1.5%, two infection with *Rhizoctonia solani* at 3%, two infection with *Fusarium solani* and two infection with *Rhizoctonia solani* with 4.5 %, So that the effect of the extract is relatively better when its use on eggplant. *Fusarium oxysporum* was unable to infect both eggplant and tomato when the extract was used in the three concentrations. When the extract was adding together with the addition of fungi to the pots (Table 4), one eggplant plant was infected with concentrations of 3 and 4.5%, for *Fusarium oxysporum* and *F. solani*. On tomato plants, there was one infected plant at 1.5% concentration and two infected plants at 3% with *F. oxysporum*, tow infected plants with concentration of 3% and 4.5% with *F. solani*. *Rhizoctonia solani* was unable to infect both eggplant and tomato when the extract was used in the three concentrations. There was also no infection on the eggplant

Table 2. The percentage of inhibition of the conocarpus extract on the growth of *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Rhizoctonia solani* (RS) in the laboratory.

Treatments	The percentage (%) of inhibition on PDA after the period/ days														
	1*			2			3			4			5		
	FO	FS	RS	FO	FS	RS	FO	FS	RS	FO	FS	RS	FO	FS	RS
T1 (0.5%)	39	-	0	42.5	-	-	26.24	-	-	24.28	-	-	25.88	6.97	-
T2 (1%)	50	-	28.5	53.8	-	-	53.68	-	20	54.7	18.18	30.3	54.94	30.23	20.86
T3 (1.5%)	61	0	57.1	57.4	18.03	8.5	57.11	20.43	26.8	57.14	24.24	34.24	62.7	41.86	52.03
T4 (The fungicide)	100	100	100	100	90.7	100	100	92.6	100	95.7	84.84	100	96.47	80.69	100

Treatments	The percentage (%) of inhibition on PDA after the period/ days														
	6			7		8		9		11		12		15	
	FO	FS	RS	FS	RS	FS	RS	FS	RS	FS	RS	FS	RS	RS	
T1 (0.5%)	20.3	-	-	-	-	-	-	-	-	0	-	-	-	1.6	
T2 (1%)	47.7	21.32	8.5	23.3	28.3	19.47	19.5	21.9	27.89	7.88	18.18	1.88	15.6	11.1	
T3 (1.5%)	64.7	46.6	57.4	52.38	66.6	56.07	66.6	56.57	72.1	61.17	74.02	66.6	75.9	77.7	
T4 (The fungicide)	96.6	81.13	100	84.12	100	80.96	100	79.45	100	76.47	93.5	77.7	93.97	94.4	

Table 3. Effect of adding the conocarpus extract after 10 days of *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Rhizoctonia solani* (RS) were added on tomato and eggplant plants in the plastic house

Treatments	Egg plants								Tomato							
	1.5 (%)		3 (%)		4.5 (%)		Fungicide (1 ml^{-1})		1.5 (%)		3 (%)		4.5 (%)		Fungicide (1 ml^{-1})	
	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
FO	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3
FS	0	3	0	3	0	3	0	3	1	2	0	3	2	1	1	2
RS	1	2	0	3	0	3	0	3	0	3	2	1	2	1	0	3
Total	1	8	0	9	0	9	0	9	1	8	2	7	4	5	1	8
Total	1 infected plant and 26 healthy plants						0		7 infected plant and 20 healthy plants						1	
Percentage of infection	4						0		25						11	

plants when using the fungicide at 1 ml l^{-1} concentration while one tomato plant was infected. When calculating the total number of infected plants in both cases, the infection rate was 4% on the eggplant plants when using the extract after 10 days of addition of fungi, and 15% when fungi and extract added together, while the rate of infection was 26% on tomato plants for the two methods, so the extract has provided protection for eggplant plants by 96% and 85% for both methods and protection of 74% of tomato plants in both methods.

Thus the eradicate method was better with eggplant plants compared with the protective method, while there is no difference between the two methods on tomato plants, the extract was effective on *F. oxysporum* in eradicate method compared with other two fungi, and the protective method benefited with the *R. solani* compared With the other two

Table 4. Effect of adding the conocarpus extract after *Fusarium oxysporum* (FO), *Fusarium solani* (FS) and *Rhizoctonia solani* (RS) were added on tomato and eggplant plants in the plastic house

Treatments	Egg plants								Tomato							
	1.5 (%)		3 (%)		4.5 (%)		Fungicide (1 ml^{-1})		1.5 (%)		3 (%)		4.5 (%)		Fungicide (1 ml^{-1})	
	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy	Infected	Healthy
FO	0	3	1	2	1	2	0	3	1	2	2	1	0	3	0	3
FS	0	3	1	2	1	2	0	3	0	3	2	1	2	1	1	2
RS	0	3	0	3	0	3	0	3	0	3	0	3	0	3	0	3
Total	0	9	2	7	2	7	0	9	1	8	4	5	2	7	1	8
Total	4 infected plant and 23 healthy plants						0		7 infected plant and 20 healthy plants						1	
Percentage of infection	15						0		26						11	

Table 5. Effect of conocarpus extract and killing ratio according to the Henderson-Tilton equation - on *Myzus persicae* (Sulzer 1776) in the laboratory on tomato and eggplant

Treatments	*Number of live insects and percentage of killing and duration after treatment days^{-1}											
	One day				Two days				Three days			
	Tomato		Egg plant		Tomato		Egg plant		Tomato		Egg plant	
	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)
T1 (1.5%)	4.3	19	5.7	0.0	3.3	23	3.3	30	1.6	65	1.3	19
T2 (3%)	4	25	4.7	18	2.3	47	3.3	3.	4.6**	0.0	0.6	62
T3 (4.5%)	2.7	51	2.3	60	1	77	1	79	1	78	2.6*	-
Control	5.3		5.7		4.3		4.7		4.6		1.6	
LSD	1.3		3.43		0.94		2.6		7.87		2.87	

*The increase in the number may be due to breeding of the insects

Table 6. Effect of Conocarpus extract and killing ratio according to the Henderson-Tilton equation - on *Aphis nerii* (Boyer de Franscolombe1841) in the laboratory

Treatments	Mortality of *A. nerri* days after treatment							
	One day		Two days		Three days		Four days	
	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)	Number	Killed (%)
T1 (1.5%)	29.7	-	31.7	16	42.7**	-	50**	-
T2 (3%)	19.3	22	14.7	61	13.3	66	18.7**	50
T3 (4.5%)	4	84	3	92	0.7	98	0.3	99
Control	24.7		37.7		39.7		37	
LSD	13.4		15.5		24.9		19.98	

*The increase in the number may be due to breeding of the insects

fungi. The allelopathic effect of the leaves of *Conocarpus lancifolius* on some plants (corn and beans) and some soil fungi was observed by Aisha Al-Shatti et al (2014) and concluded that the extract inhibits germination, root length, root and ratoon growth, chlorophyll content, photosynthesis and nutrients of the crops mentioned.

Effect of the extract of conocarpus on some insects in the laboratory: *Myzus persicae* (Sulzer 1776): There was no effect of the high concentration of extract on *M. persicae* for tomato and eggplant plants after 3 days of treatment. The extract of the conocarpus plant showed the after the first day at 4.5% concentration. The effect was significant on this insect after two days of treatment at 4.5% on the tomato and eggplant. The highest moortality was on the eggplant plant after two days of treatment was 79%, on tomato plants, and 78% after three days. There has been an increase in the number of insects after this period may be due to the breeding of the insect and degradation of active molecules gradually.

***Aphis nerii* (Boyer de Franscolombe 1841):** There was significant effect of the conocarpus extract on *Aphis nerii* e after one day of treatment at concentration of 3 and 4.5%, but there is a r superiority of the concentration of 4.5% in the killing of insect reached a maximum of 4 days after the treatment. The mortality rate was 99% after four days of the concentration of 4.5%, so the effect of the extract is higher on *A. nerii* compared to the *M. persicae*. Osman (2016) observed that *conocarpus lancifolius* extract was not effective on the mealy bug in cotton (*Phenacoccus solenopsis*).

CONCLUSIONS

The extract of conocarpus leaves in inhibit the growth of *Fusarium oxysporum*, *Fusarium solani* and *Rhizoctonia solani* at oncentration of 1.5% in the laboratory and 4.5% concentration in the plastic house. The extract of the conocarpus leaves can be used in the eradicate method against *Fusarium oxysporum* to protect the tomato and eggplant plants with high efficiency in greenhouse. The extract can also be used in a protective method to control the *Rhizoctonia solani* on the tomato and eggplant plants with high efficiency. The extract of the conocarpus were effective against *M. persicae* on the tomato and eggplant plant and *A. nerii* in the laboratory with high efficiency.

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