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ORIGINAL ARTICLE

ISOLATION AND IDENTIFICATION OF FUNGI ASSOCIATED WITH SEEDLINGS ROOTS OF *CITRUS AURANTIUM* AND ENHANCE ITS GROWTH BY USING SOME ORGANIC EXTRACTS

Hayder Ibadi Naser AL-Isawi

Department of Plant Production Techniques, Technical Institute of Kufa, Al-Furat Al-Awsat Technical University, Iraq.
E-mail: Haider-alisawi@atu.edu.iq

Abstract: This study was conducted in plant pathology laboratory and lath house of Department of Plant Production Techniques, Technical Institute of Kufa, Al-Furat Al-Awsat Technical University to isolate and identify fungi that associated with Citrus seedlings roots and improve its growth by using some organic extracts. Results showed that many fungi isolated from citrus seedlings roots as the species *Fusarium solani* achieved highest percentage of frequency and prevalence 90.91 and 43.01% respectively among all isolated fungi, while, *Arthrinium phaeospermum*, *Cladosporium sphaerospermum* and *Penicillium italicum* recorded the lowest frequency and prevalence amounted 4.55 and 1.08% respectively. The effect of some organic extracts and captin pesticide on the growth of *F. solani* and vegetative indicators of Narang was studied and the results indicated that captin was more effective in controlling the pathogenic fungus by inhibiting its growth to 0 cm followed by humic acid 5.4 cm and humic acid ground 7.1 cm in comparison with 8.5 cm in control treatment. Foliar humic acid significantly increased all studied traits but *F. solani* + captin recorded the lowest vegetative indicators as compared to positive and negative controls.

Key words: *Citrus aurantium*, Organic extracts, Captin, Chuail extract.

Cite this article

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1. Introduction

Citrus aurantium is an important fruit tree and is known for its medicinal value worldwide as all plant parts used to treat wide range of diseases [Periyanayagam *et al*. (2013)]. The deterioration of citrus (cv. Narang) trees and reducing its productivity occur due to the infection of pests and pathogens including fungi that cause many diseases such as dry root rot which causes by *Fusarium solani* [Spina *et al*. (2008)]. *F. solani* can remain in soil for many years and causes many diseases such as roots rot, wilt and slow decline diseases in citrus [Al-Sadi *et al*. (2014)]. Organic cultivation is an alternative system for mineral fertilizers which cause environmental pollution and the modern cultivation has depended on the organic methods to provide plant needs to grow in a sustainable and environment-friendly system [Willer *et al*. (2008)]. Humic acid (HA) works as biological alarm and promotes plant growth by increasing organic contents in the soil [Canellas and Olivares (2014)]. The sea algae extract (Tecamin algae) has also a role to improve plant growth by providing the necessary nutrients as well as plant hormones such as auxins, gibberellins and cytokinines which stimulate the growth of roots, increase stem thickness and total vegetative [Khan *et al*. (2009)]. The use of *Cressa cretica* plant (Chuail) extract as antivirus and germs has contributed to protect plans from many diseases as it contains coumarin, sterol, alkalis, glycosides, proteins carbohydrates and flavonoid, in addition to its toxicity for cells in general [Al-Snafi (2016)]. Thus, current study aims to isolate and identify fungi that associated with Citrus seedlings roots and

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improve its growth by using some organic extracts.

2. Materials and Methods

2.1 Isolation of fungi from roots of citrus seedlings

The isolation process was carried out by sampling the roots of six months citrus seedlings and washing it with water to remove soil and impurities, and then roots were cut into 0.5-1 cm pieces using a sterile scalpel. Samples were divided into two groups, the first group was not sterilized and the second group was sterilized by 2% sodium hypochlorite (NaoCl) for two minutes then washed with sterile distilled water and dried by putting these pieces between two layers of blotting paper. 5 root pieces were put in each petri plate contain 15-20 ml of potato dextrose agar (PDA) with 250 mg/L of chloramphenicol then plates were incubated at 25 ± 2^0C for three days and purification was performed to obtain pure cultures for diagnosis.

2.2 Diagnosis of fungi

Diagnosis was done using combined microscope and classification keys [John and Ailsa (2009), Watanabe (1937)] by determining the phenotypic and microscopic characteristics of fungi. Then the percentage of occurrence and frequency was calculated as follows:

$$\text{The percentage of occurrence} = \frac{\text{The number of samples in which the fungus appeared}}{\text{The total number of samples}} \times 100$$

$$\text{The percentage of frequency} = \frac{\text{The number of fungus colonies in all samples}}{\text{The total number of samples}} \times 100$$

2.3 The effect of organic extracts and captin pesticide on the growth of F. solani

A series of experiments were done on *F. solani* as it is the most occurred and frequent fungus isolated from citrus seedling roots. Different concentrations of studied factors were used as Tecamin and Captin pesticide used in 500, 1000, 1500 and 2000 ppm and ground humic acid, foliar humic acid were used in 150, 300, 450 and 600 ppm, Chuail extract 1:1, 1:2, 1:3 and 1:4 v:v by adding the concentrations of the above factors to PDA medium after autoclaving it on petri plates then plates inoculated with 5 mm disc of *F. solani* colony and plates were incubated at 25 ± 2^oC then data were recorded after 7 days.

2.4 Evaluate the efficiency of organic extracts and captin pesticide on citrus seedlings growth

The following traits were applied

1. Sterile distilled water only.
2. Spore suspension of *F. solani*.
3. Foliar humic acid 100 ppm.
4. Ground humic acid 100 ppm.
5. Tecamin algae 750 ppm.
6. *Cressa cretica* (Chuail extract) 1:2 v/v.
7. Captin 1000 ppm.
8. Foliar humic acid 100 ppm + Spore suspension of F. solani.
9. Ground humic acid 100 ppm + Spore suspension of F. solani.
10. Tecamin algae 750 ppm + Spore suspension of *F. solani*.
11. Chuail extract 1:2 v/v + Spore suspension of *F. solani*.
12. Captin 1000 ppm + Spore suspension of *F. solani*.

The following growth indicators were calculated

1. Leaves content of chlorophyll.
2. Number of leaves.
3. The number of branches in each plant.
4. Stem diameter (cm).
5. Leave area (cm^2).
6. Plant length before and after treatment.

2.5 Evaluate the efficiency of organic extracts and captin pesticide on the average of fresh and dry weight of total vegetative and roots of citrus seedlings

The same traits above were used and the average of fresh and dry weight of total vegetative and roots of citrus seedlings were calculated.

3. Results and Discussion

3.1 Isolate and diagnose fungi associated with citrus seedling roots

Results of isolation showed that there were many fungi associated with citrus seedlings roots including *F.*

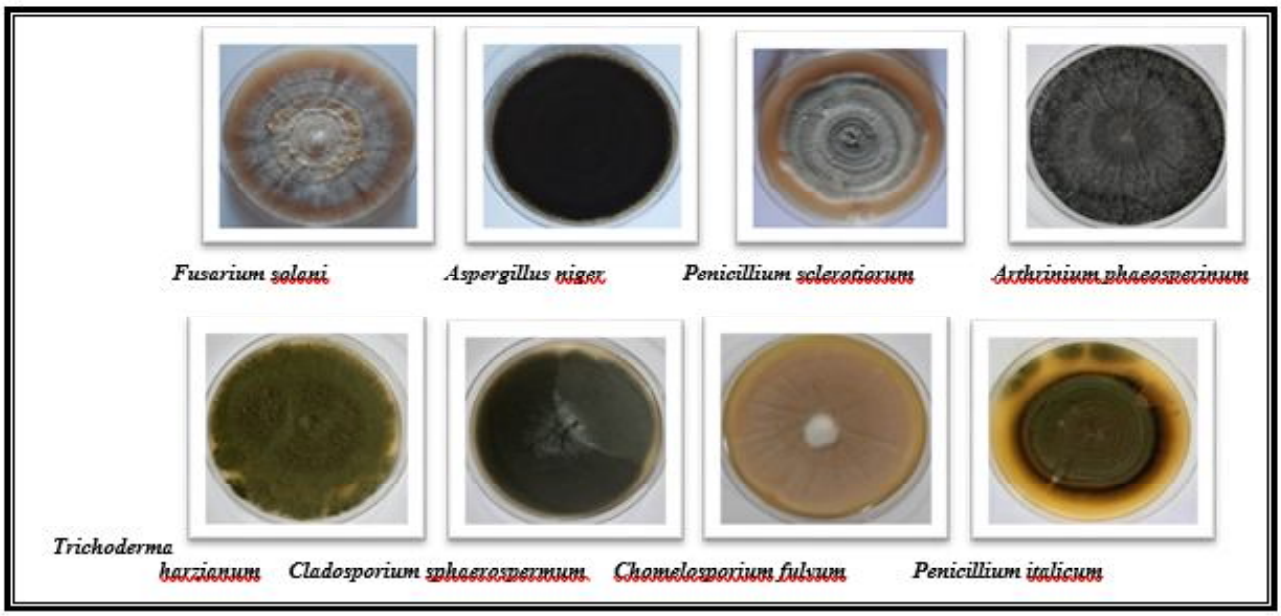

Fig 1: Isolated fungi from citrus seedling roots

solani, which its colonies appeared with white mycillium on PDA and has concentric cream rings, gray sporodochia, a lot of thick macroconidia divided with 3-4 septa and oval micro conidia or kidney shaped produced on long phialides, chlamydospores produced as single or pairs (Fig. 1). *Aspergillus niger* colony covered the whole plate and produced black conidiosporese densely, the conidiophore end with vesicule that carry phialide and conidia. *Penicillium sclerotiorum*, the colony is curly with white edges produces gray or greenish gray conidiospores. *Arthrinium phaeosperinum* covered the whole plate after 7 days at 25^0C, mycillium consists of woolen tufts which appear white or gray, the size of conidia is 10 micro meter and known as *Papularia*. *Trichoderma harzianum* cover the plate within in two days, mycilium is white or yellow with green conidia on the surface of colony. *Cladosporium sphaerospermum*, the colony has a velvety surface; the conidiophore has branches which carry the conidia. *Chomelosporium fulvum*, grow very fast and cover the whole plate, the conidiophore has one or two branches and carry the conidia mass at sterigmata. *Penicillium italicum*, the mycilium is white, produce a lot of gray or greenish conidia, phialides are 10-14 mm and the conidiophore has a cylinder shape.

3.2 The percentage of occurrence and frequency of isolated fungi from *citrus aurantium* seedling roots

The fungi that isolated from citrus seedling roots, as *F. solani* recorded the highest percentage of occurrence and frequency amounted 90.91 and 43.01% respectively among all isolated fungi, while, *Arthrinium phaeospermum*, *Cladosporium sphaerospermum* and *Penicillium italicum* recorded the lowest frequency and prevalence amounted 4.55 and 1.08% respectively (Table 1 and Figs. 2 & 3). Al-Sadi *et al*. (2014) mentioned that among 19 genera of fungi isolated from citrus roots, *F. solani* was the most occurrent and frequent.

3.3 The effect of organic extracts and captin pesticide on the growth of F. solani

Results of Table 2 showed that the concentrations of studied factors reduced the growth of *F. solani* significantly which recorded 6.01, 5.45 and 5.25 cm respectively except concentration one which amounted

Table 1: The percentage of occurrence and frequency of fungi isolated from citrus aurantium seedling roots.

Fungi	Occurrence %	Frequency %
Fusarium solani	90.91	43.01
Aspergillu sniger	40.9	24.73
Penicillium sclerotiorum	18.18	4.31
Arthrinium phaeosperinum	4.55	1.08
Trichoderma harzianum	22.73	17.2
Cladosporium sphaerospermum	4.55	1.08
Chomelosporium fulvum	31.82	7.51
Penicillium italicum	4.55	1.08

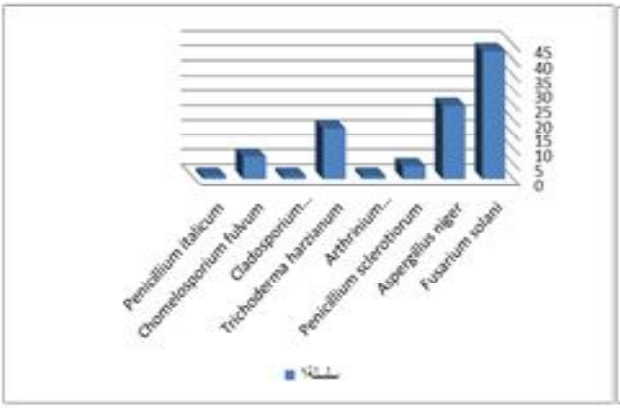

Fig. 2: The percentage of occurrence

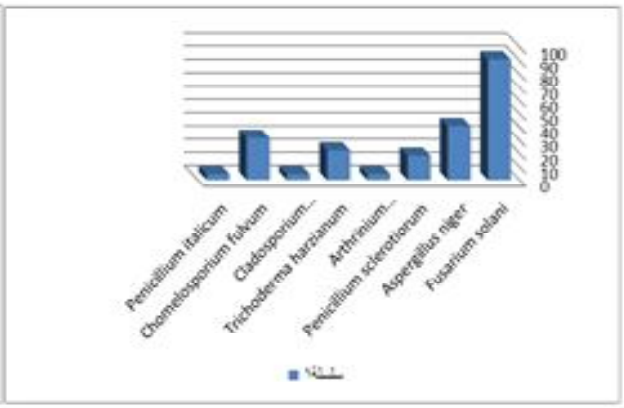

Fig. 3: The percentage of frequency

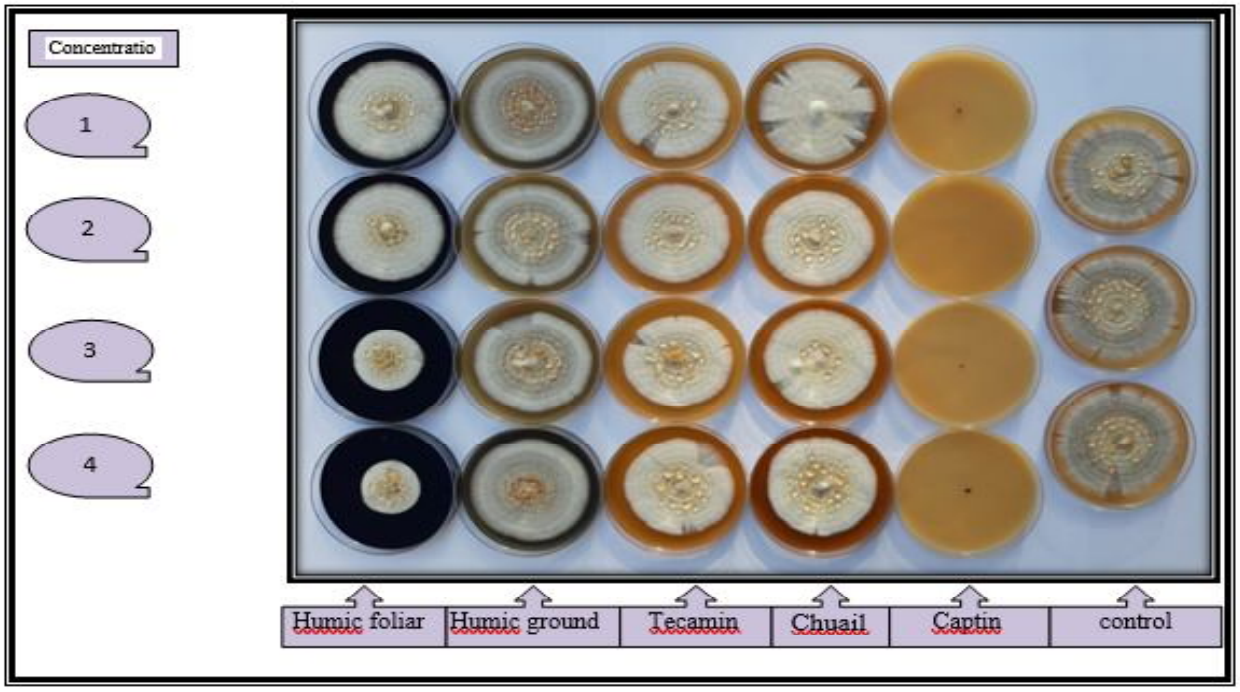

Fig. 4: The effect of organic extracts and captin pesticide on the growth of F. solani

Table 2: The effect of organic extracts and captin pesticide on the growth of F. solani.

Diameter growth the fungus F.solani (cm)					
Concentrations Treatments	**1**	**2**	**3**	**4**	**Average Treatments**
Humic acid foliar	7.1	6.7	4.2	3.6	5.4
Humic acid ground	7.7	7	6.9	6.6	7.1
Tecamin algae	6.9	6.7	6.5	6.3	6.6
Chuail extract	7.8	7.2	6.6	6.5	7
Captin	0	0	0	0	0
control	8.5	8.5	8.5	8.5	8.5
Average concentrations	6.33	6.01	5.45	5.25	
Treatments = 1.05 interaction = 2.132.31 = Concentration L.S.D. 0.05					

Table 3: The effect of organic extracts and captin pesticide on the growth indicators of Citrus aurantium.

Treatments	Leaf chlorophyll content	No. Leaf	No. of branches /plant	Stalk diameter (cm)	Leaf area cm^2	Plant lengths (cm)	
						Before treatment	After treatment
Control	28.5	72	14	2.1	18.5	70	76
Fusariumsolani	16.1	63	8	2	12.8	66	69
Humic acid foliar	49.7	152	26	3.4	25.7	80	96
Humic acid grauond	43.8	143	24	3	24.3	78	84
Tecamin alga	52.2	126	22	2.6	23.5	72	80
Chuail extract	40.1	133	23	2.9	23.2	68	79
Captin	37.2	130	18	2.7	20.4	55	67
Hu f + F.solani	42.1	139	25	3	23.6	85	90
Hu g + F.solani	47.3	121	21	2.6	22.2	73	82
Tecamin + F.solani	46.5	117	20	2.5	21.1	79	88
Chuail extract +F.solani	38.4	134	24	2.8	22.9	80	86
F.solani+ Captin	29.7	106	16	2.2	19.9	82	85
L.S.D. 0.05	4.214	5.187	2.364	1.134	2.875	3.939	4.561

Table 4: The effect of organic extracts and captin pesticide on the average of fresh and dry weight of total vegetative and roots of Citrus aurantium seedlings.

Treatments	Total weight		Total weight	
	Soft	Dry	Soft	Dry
Control	44.13	21.93	18.89	10.16
Fusariumsolani	31.44	20.75	15.32	9.88
Humic acid foliar	82.26	47.72	44.73	25.33
Humic acid grauond	0.8381	44.54	43.89	24.71
Tecamin alga	71.51	39.11	34.1	21.99
Chuail extract	59.42	25.88	28.65	19.12
Captin	47.55	23.24	26.85	17.55
Hu f + F.solani	80.76	44.73	42	23.11
Hu g + F.solani	80.33	43.67	41.55	22.86
Tecamin + F.solani	69.14	38.64	33.33	20.89
Chuail extract +F.solani	62.38	35.91	41.56	19.99
F.solani+ Captin	45.99	22.87	19.77	12.1
L.S.D. 0.05	5.255	4.141	5.354	4.421

*Each number in the Table presents the average of three replicates.

6.33 cm compared to 8.5 cm in control treatment. The captin pesticide treatment significantly excelled other treatments and inhibited the growth of fungus completely followed by foliar humic acid treatment which recorded 5.4 cm, while ground humic acid treatment recorded the lowest effect (7.1cm) on fungus growth in comparison with 8.5 cm in control. The interaction between studied factors and its concentrations on the growth of *F. solani* showed that captin pesticide treatment achieved complete inhibition, while concentration one of chuail extract was the lowest effect on the fungus growth amounted 7.8 cm compared to 8.5 cm in control.

The reason for the effect of organic extracts and reducing *F. solani* growth may occur due to the role of these extracts to change the pH of the medium that the fungus grow in and make it less acidity which is not preferred by fungi. The increasing of organic extracts concentrations led to reduce fungi growth and promote plant growth and considered safe and friendly-environment.

1. Each number in the table represents the average of three replicates.
2. Concentrations (1, 2, 3 and 4) mean

*Tecamin algae and Captin (500, 1000, 1500, 2000

ppm respectively).

*Humic acid, foliar and ground (150, 300, 450, 600 ppm respectively).

*Chuail extract (1:1, 1:2, 1:3, 1:4 v/v respectively).

3.4 The effect of organic extracts and captin pesticide on the growth indicators of Citrus aurantium

Results of Table 3 showed the effect of organic extracts and captin pesticide on growth indicators of citrus seedlings (the percentage of chlorophyll, number of leaves, number of branches in each plant, leave area, plant length after and before treatment), as foliar humic acid treatment excelled other treatments significantly and recorded 49.7, 152, 26, 3.4, 25.7, 80 and 96 respectively. While, captin + *F. solani* treatment was the lowest effect on growth indicators amounted 29.7, 106, 16, 2.2, 19.9, 82, 85 respectively compared to positive control treatment which amounted 16.1, 63, 8, 2.0, 12.8, 66, and 69 respectively and negative control which amounted 28.5, 72, 14, 2.1, 18.5, 70 and 76 respectively. The effect of humic acid may be due to the availability of iron to cover plant needs [Colombo *et al*. (2014)], and the increasing of growth indicators when using sea algae extract may occur due to its content of nutrients, plant hormones, auxins, gibberellins and cytokinines which stimulate the growth indicators [Stirk *et al*. (2003)]. The positive effect of chuail extract occurred due to its content of biologically active compounds such as coumarin, sterol, alkalis, glycosides, proteins carbohydrates and flavonoid, which can play significant role in increasing growth indicators [Al-Snafi (2016)].

3.5 The effect of organic extracts and captin pesticide on the average of fresh and dry weight of total vegetative and roots of Citrus aurantium seedlings

Table 4 showed the effect of organic extracts and captin pesticide on the average of fresh and dry weight of total vegetative and roots, when foliar humic acid treatment excelled other treatments and increased fresh and dry weight of total vegetative and roots which amounted 82.26, 47.72, 44.73 and 25.33 respectively followed by ground humic acid with 81.83, 44.54, 43.89 and 24.71 respectively. While, captin + F. solani treatment had the lowest effect and amounted 45.99, 22.87, 19.77 and 12.10 respectively compared to positive control which recorded 31.44, 20.75, 15.32 and 9.88 respectively and the negative control which amounted 44.13, 21.93, 18.89 and 10.16 respectively. Billard *et al.* (2013) found that humic acid provide iron to cover plant needs and induce the growth in leaves and roots. The increasing of roots growth when using sea algae extract related to calcium which activate enzymes that responsible for making amino acids, proteins, sugars and energy compound (ATP) which led to increase plant growth [Martin (2012)].

4. Conclusion

The organic extracts can be used to achieve plant requirements as well as protect plants from wide range of pathogens by changing the pH of the medium that fungi live in which indirectly affect these pathogens. In addition, organic extracts is friendly environment and cause less pollution to soil and water.

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