International Journal of Multidisciplinary Research and Growth Evaluation
ISSN: 2582-7138
Received: 21-09-2021; Accepted: 04-10-2021
www.allmultidisciplinaryjournal.com
Volume 2; Issue 6; November-December 2021; Page No. 49-53

Phytochemical analysis and antifungal activities of alcoholic extract of *Curcuma longa* rhizome

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Abstract
The study aimed to test the inhibitory activity of the alcoholic extract of turmeric, extract the active compound curcumin then diagnose it by using high performance liquid chromatography (HPLC), and compare its inhibitory activity with the alcoholic extract of turmeric against some types of fungi *Fusarium* spp. Where it was found that the alcoholic extract of the turmeric plant *Curcuma longa* had a slight significant effect against the tested isolates grown on the culture media Potato Dextrose Agar PDA, as measured by the inhibition percentage of isolates treated with curcumin extract at a probability level of 1%. The percentage of isolates of *Fusarium tricinctum* treated with alcoholic extract of turmeric was 54.73%. While the inhibition rate of the same isolate treated with curcumin extract was 75.66% at a concentration of 6 mg/ml, and the percentage of inhibition in the growth rate of fungi colonies treated with alcoholic extract of turmeric was 34.03%, *Fusarium solani*. While the inhibition rate in the average colony diameters of the same isolate treated with curcumin extract was 84.40%. Also, some active compounds were detected in the alcoholic extract of turmeric, each of which contained phenols and flavonoids, and their content of phenols was 689.5 mg/g for turmeric, while the percentage of flavonoids was 489.8 mg/g for turmeric. In testing the effect of copper oxide nanoparticles CuO NPs against the colony growth of *Fusarium* isolates, it was noted that there were no restrictions in the radial growth of colonies at all the concentrations used, but there was a decrease in the fungal density of *F.tricinctum* isolate colonies, and the color change of *Fusarium solani* colonies was also observed.

Keywords: *Fusarium*, *Curcuma longa*, curcumin, HPLC, CuoNPs

Introduction
Plants serve humans as a source of food and medicine, but these plants are exposed to many pathogens such as insect pests, nematodes, bacteria and fungi, causing a range of diseases (Farooq *et al*., 2010) [18]. *Fusarium* are one of most important economically important plant pathogens and widespread and has a wide family range as it affects industrial crops, fruit crops, legumes, ornamentals, vegetable crops, grain crops and forests with various types of diseases such as wilt, stem rot, root rot, fruit rot, blight and cankers (Brown and Proctor, 2013; Zubi, 2016) [10]. The fungus is also characterized by its ability to produce mycotoxins in the field and during storage, these toxins lead to contamination of food and feed (Sui *et al*., 2014; Zubi, 2016), meaning that its harm is not only limited to yield losses, crop damage and reducing their quality, but also humans and animals (Bakker *et al*., 2018; Abdel-Azeem *et al*., 2019) [7, 4]. Therefore, it was necessary to protect humans, animals and plants from these pathogens and their toxins by resorting to the use of chemical pesticides, which achieved satisfactory results in improving agricultural production. However, the treatment using these pesticides showed side effects on the environment, as these substances are characterized by the property of accumulating in the soil, causing the death of a large number of soil organisms, in addition to their leakage into water sources, which leads to damage to aquatic organisms, and they are transmitted through the food chain to other living organisms (Yadav *et al*., 2015; Kumar, 2018) [22] and also affected human and animal health due to the persistence of its effects on food (Tao *et al*., 2020) Therefore, the demand for agricultural crops that are not treated with pesticides has increased, as they are in great demand globally, so studies have tended to search for alternatives to chemical pesticides in several directions, including biological control using microorganisms (such as bacteria and fungi) and the use of extracts of medicinal plants known for their inhibitory effect on fungi because these plants contain many effective compounds Such as phenols, flavonoids, alkaloids and quinones, which are characterized by being photo-inhibiting substances singly or synergistically to enhance its bioactivity (Boga *et al*., 2021) [8].

Studies have also recently directed towards the use of nanotechnology in agricultural systems, using nanoparticles engineered for semi-metals and metal oxides, whose dimensions range between 1-100 nanometers, and due to their small size, large surface area and high reactivity, which allowed them to be used as fungicides and nano fertilizers. Alleviate challenges in disease management by reducing chemical inputs to agricultural systems (Elmer and White, 2018) [16]. The study aimed to evaluate the inhibitory activity of turmeric alcohol extract and curcumin extract, as well as the efficiency of copper oxide nanoparticles against Fusarium spp.

Materials and working methods

- **Sample source**

A field survey was conducted to investigate the types of fungi Fusarium spp from the roots of ornamental plants and vegetable crops from different regions of the city of Mosul during the months (August, September, October and November) of the year 2020, and the plants were selected based on the pathological symptoms appearing on them, such as yellowing of leaves and death of branches, noting the discoloration of the vascular bundles in the root and stem in brown When cut longitudinally with a knife, then placed in nylon bags and transferred to the laboratory and diagnosed based on their phenotypic and microscopic characteristics according to the approved taxonomic keys (Booth, 1971; Leslie and Summerell, 2006) [9].

- **Preparation of the alcoholic extract**

I followed the method Grand *et al*., (1988) [19] modified by the method of researcher Verpoorte, (1982) to prepare the alcoholic extract of the turmeric plant Curcuma longa by mixing 20g of the plant's powder and dissolving it in 200 ml of ethanol alcohol (95%) in an ice bath, then shaking the mixture well with an electric shaker, and leaving Refrigerate at 4°C for 72 hours. The mixture is filtered through several layers of gauze, then passed through a Buechner funnel and placed in a rotary evaporator at 40°C to dry and then kept in special bottles at 4°C until tests are performed.

- **Testing the effect of alcoholic extract of turmeric on *Fusaruim* genus**

Taking 1g of the crude plant extract prepared in paragraph (1), dissolved in 5 ml of (DMSO) Dimethyl sulfoxide and sterilized the mixture by pasteurization at a temperature of 63 °C for 10 minutes. The concentrations of 2, 4, 6, 8 mg/ml of the extract were prepared with PDA culture medium to obtain at the lowest concentration of MIC inhibitor, the comparison treatment was prepared from culture media with distilled water and with three replications for each treatment then the dishes were inoculated with a disc diameter 5mm and then incubated at a temperature of 25 ∓ 2 C for five days, the results were taken by calculating the average of two perpendicular diameters of the growing colonies.

$$\text{Percentage of inhibition} = \frac{\textit{Average comarion diameter} - \textit{Average diameter of the comparison colony}}{\textit{Average diameter of the comparison colony}} \times 100$$

- **Estimation of the total content of phenols**

The total content of phenols in the ethanolic extract of turmeric was estimated according to the method presented by Chang *et al*., (2002) [12], a method using Gallic acid and Folin-Ciocalteu reagent, the absorbance value was recorded at the wavelength 765 nm, and the total phenol content was calculated relative to the titration curve of gallic acid and in units of mg/g dry weight.

- **Estimation of the total content of flavonoids**

The total content of flavonoids in the ethanolic extract of turmeric was estimated according to the method presented by (Kaur and Kappor, (2002) [20] It is a colorimetry method for aluminum chloride in the presence of a substance. The absorbance was measured at the wavelength 510 nm and the total flavonoid content was calculated with respect to the calibration curve of Rutin and in units of mg/g dry weight.

- **Prepare curcumin extract**

Preparing the extract by taking0.25 g of turmeric root powder *Curcuma longa* was placed in a 250 ml volumetric flask to which 100 ml of acetonitrile and 2 ml of phosphoric acid were gradually added and mixed with an ultrasonic device for 10 minutes, then the sample was filtered with Wattman1 filter papers and then kept in opaque glass bottles until HPLC analysis and testing.

- **Diagnosis of curcumin by high-performance liquid chromatography HPLC**

The curcumin compound was diagnosed by a high-performance liquid chromatography device (HPLC SYKMN of German origin, with a flow rate of 1.2 ml/min).

The compound was detected at a wavelength of 425 nm.

Test Effect of curcumin extract on some species of the *Fusaruim* genus

To test the effect of curcumin extract, first sterilize the extract by pasteurization at a temperature (63-64) C for 10 minutes, then the concentrations of (2, 4, 6) mg/ml of the extract were prepared with the nutrient medium to obtain the lowest concentration of MIC inhibitor. The comparison treatment was also prepared from the nutrient medium with distilled water added to it, and with three replicates for each treatment, inoculated dishes with a diameter of 5 mm from the edge of a pure fungal colony, and incubated at a temperature of (25± 2) C for 5 days, and the results were collected by calculating the average measurement of each two perpendicular diameters of the growing colony and then calculating the percentage of inhibition as mentioned previously.

- **Chemical control using copper oxide nanoparticles CuONPs**

Test the effect of copper oxide nanoparticles CuONPs (of 99% purity, 6 g/cm density, 30-50 nm diameter), was taken 250 mg and dissolved in 10 ml of deionized water (Al-Issawi and thalg, 2020) and prepared concentrations of 2, 4, 6, 8 mg/ml solution with the food medium PDA to obtain the lowest inhibitory concentration, as the comparison treatment was prepared from the culture medium with distilled water added to it, and with three replications for each treatment. , inoculated dishes with a diameter of 5 mm for each fungus and incubated at a temperature of (25± 2) C for 5 day then got the results.

- **Statistical Analysis**

The experiment was analyzed using a completely randomized design and at a probability level 1% by electronic computer according to the SAS system programs, (2002) in the treatment experiments to find the analysis of variance in addition to obtaining the significant differences between the averages of the transactions by Duncan method for all the studied traits (Antar and Alwakaa, 2017) [2].

Results and discussion Scientific name of the plant family

- **Sample source**

The results of the field survey that were conducted in three areas of the city of Mosul during the months of August, effect on the growth rate of diameters *Fusarium* spp from the roots of some ornamental plants and vegetable crops that showed symptoms of *Fusarium* fungus diseases to isolated a number of species and five isolates were selected for testing as shown in Table (1).

Table 1: Isolates *Fusarium* fungus and their plant families isolated from them

Fungus	Scientific Name of the Plant Family
F.tricinctum	*Cestrum nocturnum*
F.verticillioides	*Solauum melongena*
F.oxysporum	*Agave attenuata*
F.solani	*Capsicum annum*
F.poae	*Tillandsia cyanea*

Effect of alcoholic extract of turmeric roots on some types of Fusarium genus

The alcoholic extract of turmeric roots showed a clear effect on the growth rate of diameters of Fusarium spp fungus species (Table 2). the clearest effect of this extract appeared on F.poae fungus isolation, where the inhibition rate in the average of its colony diameters was 72.3% at 8 mg/ml concentration, also had an effect on the growth rate of the colonies diameters of the rest of the isolates, which are F.tricinctum, F.verticillioides, F.oxysporum and F.solani, with inhibition rates of 63.2, 64.2, 52.4, 51.8%, respectively, at the same concentration. The table also shows that the rate of colonies diameters is inversely proportional to the concentration of the extract, in contrast to the percentage of inhibition, which was increased by increasing the concentration of the extract, except for isolate F. solani, which showed the lowest percentage of inhibition at a concentration of 4 mg/ml. The reason may be attributed to the fact that the fungus at this concentration showed resistance against the components of the extract, which led to the continuation of the mycelium growth.

It is clear from the above that the alcoholic extract of the roots of the turmeric has a clear effect on the growth rate of the fungus isolates Fusarium. This effectiveness may be attributed to the plant's containing effective compounds such as curcumin, phenols, flavonoids, saponins, and volatile oils such as Cineol, Sesguiterpens and Zingibaren oils, as these compounds interfere with the functioning of the cell membrane of the fungi (Chattopadhyay *et al*., 2004; Rezvanirad *et al*., 2016) [13]. These results are consistent with the results of many researches and studies that were conducted to test the effect of alcoholic extracts of medicinal plants. Al-Quraishi (2011) reported that the alcoholic extracts of turmeric roots, bark of scholars, sesame seeds, bitter melon seeds, salt leaf fruits and oleander flowers showed an inhibitory effect on some human pathogenic fungi such as T. .rubrum E.floccosum A.niger.

Chen *et al*. (2018) [15] found that the alcoholic extract of turmeric has strong antifungal activity, including F.colletotrichum, F.colomorum, F.chlamydosporum, F.graminearum, and F.oxysporum. Its antifungal effect is due to disruption of the fungal cell membrane.

Table 2: Effect of alcoholic extract of turmeric roots on average colonies diameters on types of *Fusarium*

Species fungus Fusarum	Concentration 0 mg/ml		Concentration 2 mg/ml		Concentration 4 mg/ml		Concentration 6 mg/ml		Concentration 8 mg/ml		the average	
	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	% of inhibition	Average total colony diameters	inhibition rate total
F.tricinctum	3.500 EGP	0.00 i	2.350 Zg	31,866 f g h	1.883 i j k	46.13 d	1.583 kL	54.70 AH	4.633 m	63.26 b	2.7900 dinars	39.19
F.verticillioides	4.300 AB	0.00 i	3.033 dh	29.400 g h	2.533 and g	34.53 f	1.800 i j k	58.73 b c	1.500 lm	64.26 b	2.63 b	37.3 b c
F.oxysprum	4.00 b	0.00 i	2.700 AH and	25.83 h	2.150 h i	46.23 d	1.966 i J	50.75 EGP D	1.950 i J	52.46 EGP D	2.55	35.0 EGP
F.solani	4.00 a	0.00 i	2.983 dh	33.80 f g h	3.116 D	30.70 and Zh	2.983 dه	34.03 and g c	2.166 h i	51.80 EGP D	3.15 A	30 d
F.poae	4.400 A	0.00 i	2.700 AH and	38.366 AH and	2.083 H i J	52.600 EGP	1.566 kL	63.23 b	1.216	72.300 A	2.39 EGP	45.33 A
	4.14 A	0.0 e	2.75	31.85 D	2.35 EGP	42.04 EGP	1.98 d	52.29 b	1.63 AH	a		

The numbers in the table represent the average of three replicates

Numbers followed by different letters indicate the present of significant differences within Duncan's multiple range test at the 1% probability level.

- **Total content of phenols and flavonoids**

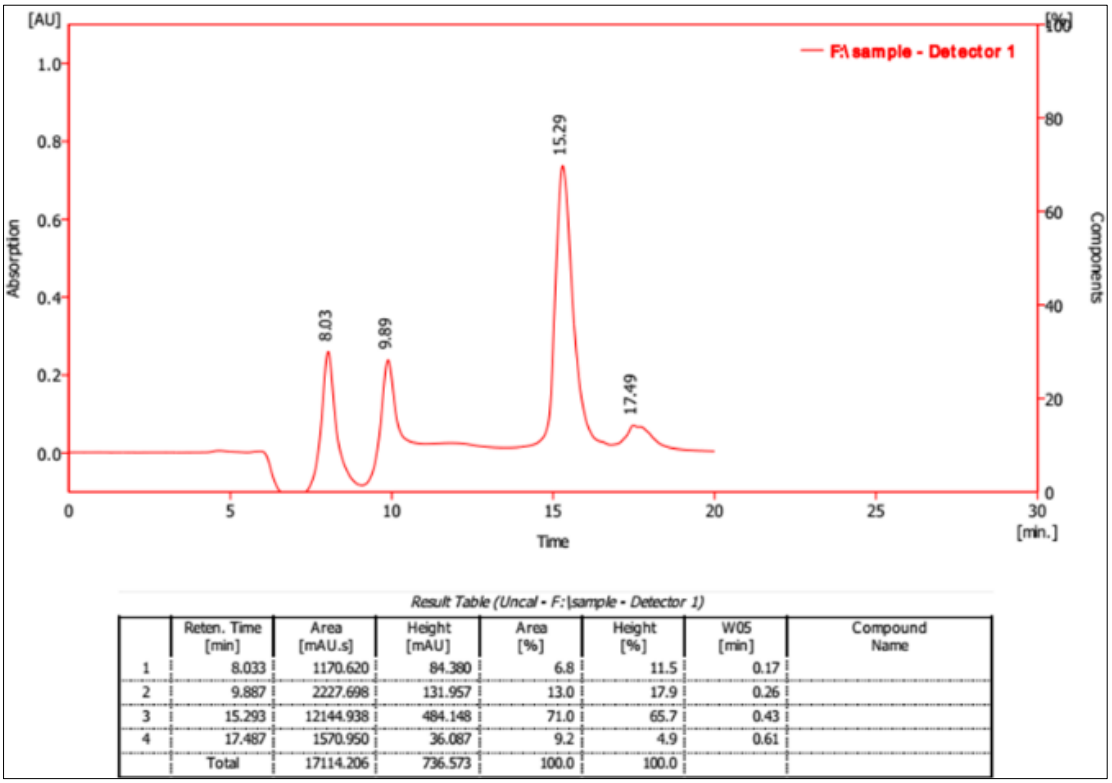
The total content of phenols and flavonoids of the ethanolic extract of turmeric was estimated as shown in the following table.

Table 3: The total content of phenols and flavonoids in the alcoholic extracts of turmeric plants.

Ethanolic extract of plant	Total content of phenols mg/gm	Total content of flavonoids mg/gm
turmeric	689.5	489.8

High performance liquid chromatography (HPLC) technology diagnosed curcumin compound

The chromatographic analysis charts were obtained, which determine the retention time of curcumin compound of 15.29 minutes (Fig. 1).



Result Table (Uncal - F:\sample - Detector 1)

	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	8.033	1170.620	84.380	6.8	11.5	0.17	
2	9.887	2227.698	131.957	13.0	17.9	0.26	
3	15.293	12144.938	484.148	71.0	65.7	0.43	
4	17.487	1570.950	36.087	9.2	4.9	0.61	
	Total	17114.206	736.573	100.0	100.0		

Fig 1: Curve of the active compound of curcumin in the roots of the turmeric

- **Effect of curcumin extract on Fusarium genus**

Curcumin extract showed a clear effect on the rate of colonies diameters of fungus species Fusarium sp where the inhibition percentage of the isolate belonging to F.solani fungus was 84.4% at the concentration (6 mg/ml), while the inhibition percentage of the isolate belonging to F.tricinctum reached (75.7%) at the same concentration (Table 3). From the results of inhibition, it was clear that there were significant differences in the growth rate of colonies diameters compared to the control treatment. The antifungal activity was attributed to the curcumin compound. These results agree with the study of Chio and Kim, (2003) that the ethyl extract (acetonitrile) of the turmeric (curcumin) showed an antifungal effect for P.infestans, R.solani at 1000 mg/L concentration are in agreement with the findings of Chawdhury *et al*., (2008). Where curcumin and turmeric oil have antifungal effects of two types of fungi, Helminthosporium oryzae, F.solani. The results also agree with a study by Apisariyakul (1995) [6] when extracted from turmeric oil and curcumin in order to test its efficacy against dermatophytes and yeasts, Where these extracts showed inhibitory activity against pathogenic dermatophytes, but ineffective against yeasts.

And in a study of Lee and Lee (2014) to test the inhibitory activity of curcumin and reveal its mechanism of action as an antifungal Candida albicans and found that it exerts an antifungal activity by disrupting the plasma membrane of the cell.

It also agrees with the study of Akter *et al*. (2018) [5] to test the efficacy of curcumin extract against F.solani fungus, which showed antifungal activity at a concentration of 25 g/ml.

Table 4: Effect of curcumin extract on the average colonies diameters of *Fusarium* fungus

types of fungus Fusaruim	Concentration 0 mg/ml		Concentration 2 mg/ml		Concentration 4 mg/ml		Concentration 6 mg/ml		The Average	
	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	% of inhibition	Colonies average	rate of inhibition
F.tricinctum	3.500 b	0.00 H	1.183 c	66,166D	1.000 H	71.40 EGP	0.85 and	75.66 b	1.63	53.31 B
F.solani	4.500 A	0.00 H	1.1333 c d	74.76 a b	1.066 DH	76.23 b	0.700 g	84.40 A	1.85 A	58.85 A
	4.0 a	0.0 d	1.16 b	70.47 EGP	1.03 c	73.82 b	0.78 d	80.03 A		

The numbers in the table represent the average of three replicates.

Numbers followed by different letters indicate the presence of significant differences within Duncan's multiple range test at the 1% probability level.

- **Particle effect copper oxide nanoparticles CuONPs against some species of Fusarium genus**

It was observed from the results of testing the effect of copper oxide nanoparticles CuoNPs when mixed with the nutrient medium: Potato dextrose agar (PDA) that there were no restrictions in the radial growth of colonies of Fusarium, but there was a decrease in the density of the mycelium of the colonies of isolate F. tricinctum

It was also observed that a change in the color of its colonies and that of the isolate F. solani may be attributed to the reason that copper oxide nanoparticles did not affect the growth rate of colonies diameters because the incubation period (5 days) was not enough, it may need a longer incubation period to show its effect on the growth rate of colonies diameters, also the role of the agar medium in the restriction, release and availability of copper ions is unknown. These results are

consistent with the results of Elmer and White (2016) [17] when they tested the inhibitory activity of copper oxide nanoparticles when mixed with the agar medium, they did not notice any change in the growth of the colony diameters of the fungus F. Oxysporum even at concentrations of up to 1000 µg/ml of CuONPs nanoparticles, in contrast to the results reached by the researchers in the year (2018). When they tested the inhibitory activity of six metal oxides, including copper oxide nanoparticles, they found that copper oxide nanoparticles were more effective in inhibiting the growth of F.oxysporum on tomato plant and Verticillium dahliae on eggplant.

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