The efficiency of some plant extracts on the fungus Macrophomina phaseolina, the causes agent of seed rot and the Okra seedlings death

The efficiency of some plant extracts on the fungus *Macrophomina phaseolina*, the causes agent of seed rot and the Okra seedlings death

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Abstract

This study included the assessment of the antifungal activity of extracts of Cuminum cyminum, Zingiber officinale and Citrullus colocynthis on the growth of Macrophomina phaseolina. Additionally, the toxicity effect of the fungal filtrate on the percentage of okra seeds germination as well as some okra parameters. The results showed that the alcoholic extract of Cuminum cyminum had a significant effect on the inhibition of the radial growth and dry weight of M. phaseolina followed by Zingiber officinale and citrullus. Among the all extracts, Cuminum cyminum extract gave the highest germination, which was 66%, while the Zingiber officinale and Citrullus colocynthis had lowest effect on the germination by 53.3 and 46.2 respectively. The concentration of 50% tends to have a significant influence on okra seeds germination 55.1% than 25% concentration. The alcoholic extract of Cuminum cyminum had a significant effect at 3% concentration on plumule height, which was 6.2 cm, while zingiber officinale and citrullus colocynthis at the same concentration (3%) had plumule heights of 5.2 and 2.8 cm respectively. Cuminum cyminum extract also had a significant effect on the radiant, which was 1.8 cm. A similar effect on the radiant was examined to zingiber officinale which was 1.5 cm. In conclusion, the Cuminum cyminum extract exhibited a promising effect on some parameters of okra seedlings, especially for high concentrations. All alcoholic extracts under investigation showed an effective effect on infected fungi, in particular, Cuminum cyminum extract.

Keywords: Antifungal, Citrullus colocynthis, Cuminum cyminum, Zingiber officinale, Okra

1.Introduction

Okra, *Hibiscus esculentus*, belongs to the Malvaceae family. Okra is one of the important vegetable crops grown in warm regions of Asia and Africa [1]. It is an important vegetable in Iraq. Okra is rich in certain nutrients such as calcium, magnesium and phosphorus. It also contains some vitamins in moderate proportions such as vitamin C and vitamin A [2,3].

Generally, okra plant is infected with several pathogenic fungi with economic effect, including *Macrophomina phaseolina*, which has wide family hosts, affecting more than 500 plant species [4]. The incidence of this fungus increases when the plant is exposed to environmental stress factors such as high temperature and low humidity [5]. Recently, researchers have shown an increased interest in reducing the use of chemical pesticides because of the health risks associated with their use [6]. Many agricultural and research institutions have recently turned to find an alternative, harmless and highly efficient methods to reduce the effect of plant pathogens [7].

The use of plant extracts is one of these methods. The use of medicinal plants has become increasingly common in recent years in several countries because of their important active substances with antimicrobial properties [8], encouraging seed growth and reducing fungal diseases [6]. Plant extracts emerged as promising alternatives to chemical resistance. The present study was carried out with the aim of determining the inhibitory efficacy of some alcoholic extracts of a group of medicinal plants against *M. phaseolina* and their interactions with the okra growth.

2.Experimental

This study was conducted at the laboratories of the Plant Protection Department, College of Agriculture, Basrah University, Basrah, Iraq.

2.1 The Plant Materials and Fungal Pathogen

Three types of medicinal plants were selected in this study include; *Cuminum cyminum*, *Zingiber officinale* and *Citrullus colocynthis*, which are available in the local markets at Basrah city.

Macrophomina phaseolina was obtained from the Plant Protection Department, College of Agriculture, Basrah University.

2.2 Preparation of alcoholic plant extracts

To crushed 50 g of each vegetable sample of the *Cuminum cyminum*, *Zingiber officinale* and *Citrullus colocynthis* in a 500 mL pre-prepared container, 250 mL of ethyl alcohol was added at 70% concentration separately. Then the mixture was stirred on a hotplate for 48 hours at a temperature 45-50 °C intermittently. The mixture was filtered and then the filtrate was placed in the centrifuge at 3000 rpm. The precipitate was removed from the filtrate and this process was repeated three times to ensure that the filtrate is clean from the precipitate. Subsequently, the filtrate was filtered using the filter paper Whatman no 1. The solution was concentrated using the electric oven at 30 °C until it becomes thick liquid and then kept in the refrigerator at 4 °C in sterile bottles [9].

2.3 The inhibitory effect of different concentrations of alcoholic plant extracts on the growth of the fungus M. phaseolina

The extracts of *Cuminum cyminum*, *Zingiber officinale* and *Citrullus colocynthis* at concentrations of 1, 2, and 3% were prepared by adding 1, 2 and 3 g / 100 ml of the sterilized PDA respectively. Each weight of these weights was dissolved in 1 ml of ethyl alcohol 70% to dissolve the extract before adding it to the sterile PDA, then the vials containing the sterilized medium were shaken to homogenize the extract. The medium then was poured in sterile glass dishes of 9 cm diameter with three replicates for each concentration of each alcoholic plant extracts. After hardening the center, each center of the dish was chopped with a 0.5 cm disc taken from the middle edge of the 7-day *M. phaseinolina* fungal culture. The dishes were incubated at 25 ± 2 °C for 7 days after which the effect of the extract was measured in the inhibition of pathogenic fungus by taking the rate of two orthogonal diameters passing at the center of the dish from the back of the culture. The percentage of inhibition was calculated according to the equation below:

$$\%\ Growth\ rate\ inhibition = \frac{\text{Fungal Growth at control} - \text{Growth at treated sample}}{\text{Fungal Growth at control}} \times 100$$

2.4 The inhibitory effect of different concentrations of alcoholic plant extracts on the dry weight of M. phaseolina

The liquid medium PD broth was prepared and sterilized by autoclave. Alcoholic extracts of *Cuminum cyminum*, *Zingiber officinale* and *Citrullus colocynthis* were added after sterilization in concentrations 1, 2 and 3% by adding 1, 2 and 3 g / 100 ml. The concentrations 1 and 2% were excluded for both *Zingiber officinale* and *Citrullus* for not giving a result in the experiment of inhibiting the fungal growth. The vials were well shaken, and some flasks were left without adding them to the comparison. The flasks were inoculated with 0.5 cm dishes taken from the edge of the 7-day pathogen colony. Each treatment was repeated three times, incubated at 28 °C for 10 days. The fungal growth was collected after the end of the incubation period by forceps and then it was dried in the oven at 80 °C for 24 hours [10]. Dry weights were calculated, and the rate of inhibition was calculated in dry weight.

2.5The toxic effect of pathogenic fungus on the germination percentage of okra seeds in dishes

M. phaseolina and the plant extracts from the previous experiment were used after filtration in the vacuum machine using 0.45 microns filter papers. 10 grams of okra seeds were sterilized with 10% chlorax solution for 5 minutes and four times with H_2O and distributed after sterilization on 9 cm diameter Petri dishes containing sterile filter papers with 15 seeds per dish. The seeds were treated with fungal colony filtrations obtained from the previous experiment with concentrations of 25 and 50%. Each treatment was repeated three times, leaving a treatment group with distilled water only for comparison. Incubate the dishes in the incubator at 25 °C for 10 days. The rate of germination was calculated, and three plants per replicate and the length of the plumule and the root was calculated and then their rates were obtained.

2.6 Effect of plant extracts on the growth of okra seedlings

2.6.1 Preparation of the fungal inoculum and mixing it with the peat moss

The fungus inoculum of *M. phaseolina* was multiply by three dishes of Petri dishes and was mixed with 3 kg of sterile peat moss. The fungal colonies growing on the PDA medium were cut by a sterile knife into small pieces and mixed well after the peat moss was treated with suitable moisture content.

2.6.2 Addition of alcoholic plant extracts and mixing them with the treated peat moss

Peat moss was divided by the fungal inoculum into the following treatments for each concentration separately:

1. Treated peat moss with pathogenic fungi + alcoholic extract of *Cuminum cyminum*
2. Treated peat moss with pathogenic fungi + alcohol extract of z*ingiber officinale*
3. Treated peat moss with pathogenic fungus + alcohol extract of *citrullus colocynthis*
4. Treated peat moss with pathogenic fungi (comparative treatment) when the effect a significant

The weight of the okra, the root length, the seedling length and the percentage of seed germination were calculated.

2.7 Statistical analysis

A complete randomized design as a factorial experiment was employed in all experiments with three replicates. The all presented results were analyzed by using the software SPSS program for windows (version 10.0). Statistical significant was confirmed by ANOVA (Analysis of variance) and with least significant difference (LSD) test at the probability level of 0.05. All results were expressed as mean after converting the percentage of the data according to arcsine conversion.

3. Results and discussions

3.1 The antifungal activity of plant extracts on the growth of pathogenic fungi

Table (1) compares the experimental data on the inhibitory effect of plant extracts on the growth of pathogenic fungi in three concentrations 1, 2 and 3%. The results of Table (1) shows the superiority of the alcoholic extract of the *Cuminum cyminum* with significant differences than *Zingiber officinale* and *citrullus colocynthis*, with a rate of inhibition of 30.3%.

The concentration of 3% showed the highest inhibitory rate of *M. phaseolina* 29.2%, while the concentration of 2% did not differ significantly from 1% with an inhibitory rate of 9.3% and 6.5%, respectively.

Additionally, Table (1) shows a significant difference between the effect of the plant extract and the concentrations used. The *Cuminum cyminum* extract at 3% had the growth inhibiting rate of 43.6% followed by the *Zingiber officinale* at 3% concentration with 29.2% inhibition. *Cuminum cyminum* superiority may be due to containing alkaloids, which have the potential to inhibit cell cytoskeletal synthesis, leading to the death of the organism [11,12]. The inhibitory effect of *Zingiber officinale*, which is the least likely effect of latency, may be due to the presence of a risomate on Gingerol, which plays a disincentive role in the growth of microorganisms [13]. Some studies have indicated that the alcohol extract of *Zingiber officinale* contains the glycosides, alkaloids and phenols, making them more effective [14].

Table 1. The inhibitory effect of different concentrations of alcoholic plant extracts in *M. phaseolina*

Plant extracts	Concentrations			Average of plant extracts
	1%	2%	3%	
Cuminum cyminum	19.5	28.06	43.6	30.3
Zingiber officinale	0	0	29.1	9.7
Citrullus colocynthis	0	0	14.9	4.9
Average of concentration	6.5	9.3	29.2	

L.S.D. 0.05 for concentrations = 3.8 L.S.D. 0.05 for plant extracts = 3.7 L.S.D. 0.05 for interaction = 2.4

3.2 The inhibitory effect of plant extracts on the dry growth of M. phaseolina

Table (2) presents the inhibitory effect of plant extracts on dry growth of *M. phaseolina*. It can be seen from the data in Table 2 that *Cuminum cyminum* extracts were effective in inhibiting the dry weight of the fungus fungi by 90%. *Zingiber officinale* and *Citrullus colocynthis* came in second place in dry weight inhibition, with the inhibition rate being 70.71 and 65.95% respectively.

The active compounds possessed by the plant extracts have inhibitory effects for the growth of the fungi which have some enzymes which destroy the wall cell by affecting some its composition leading to destroy the whole cell [15]. In 2011, Abdulhassan [16] pointed to the effect of some powdered garlic, black pepper and albizia leaves on the dry growth of the fungus *M. phaseolina*.

Table 2. The inhibitory effect of alcoholic plant extracts on the dry weight of *M. phaseolina*

Plants Extract	Growth Inhibition %
Cuminum cyminum 1%	44.98
Cuminum cyminum 2%	50.31
Cuminum cyminum 3%	90.00
Zingiber officinale	71.70
Citrullus colocynthis	65.95

L.S.D. 0.05 for plant extracts = 5.80

3.3 The toxic effect of pathogenic fungus on the germination percentage of okra seeds.

The results obtained from the experiment of the toxic effect of pathogenic fungus on the okra seeds are shown in Table (3). The results of this experiment showed that the *Cuminum cyminum* extract (3%) gave a growth rate of 66% followed by *Zingiber officinale* and *Citrullus colocynthis* at the same concentration (3%) 53.3 and 46.2% respectively. Also, the concentration of 50% had a significant effect on the percentage of growth 55.1%, while the concentration of was 25% and slightly less significant at 41.4%. The results also showed a significant interaction between plant extracts at concentrations of 50% and 25%, *Cuminum cyminum* 3% showed at concentration 50% a significant effect on growth rate which reached to 79.2%. *Zingiber officinale* and *Citrullus colocynthis* had less effect on growth at 50% which were 59.1% and 47.4% respectively.

This is consistent with Delucca et al [17], who pointed out that the increase in growth rate due to increased concentration of anti-fungal growth and the growth of more seeds.

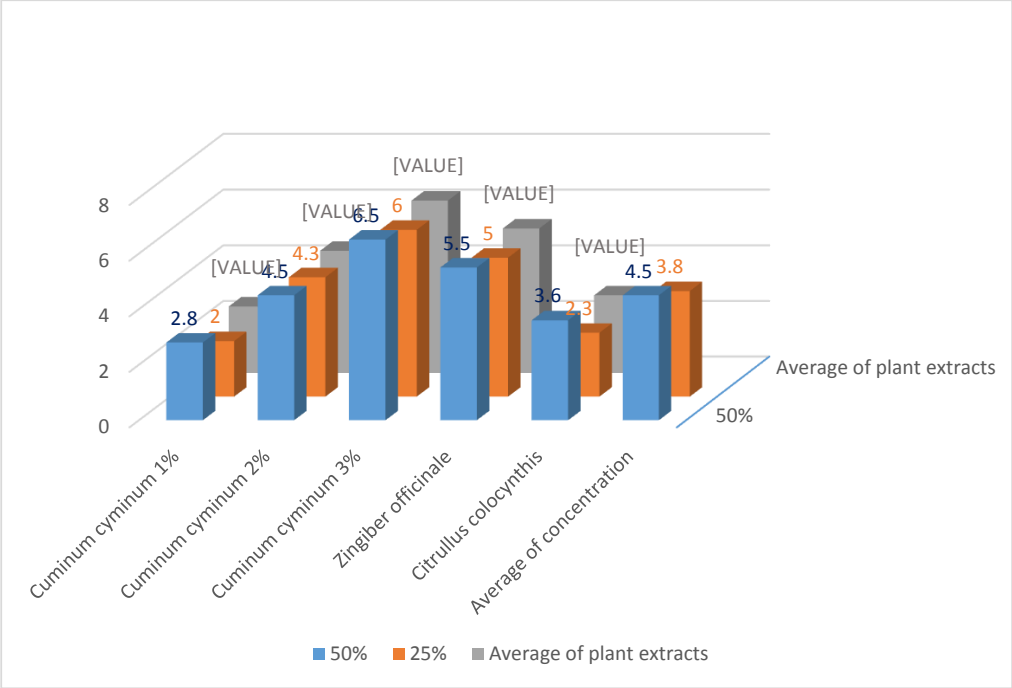
Figure (1) shows the experimental data of the toxic effect of pathogenic fungus on the length of the plumule. The results of Figure (1) showed that the 3% *Cuminum cyminum* extract had a higher effect in the length of the plumule which was 6.2 cm, while the effect of the 3% *Zingiber officinale* plant extract was 5.2 cm. There was no significant difference between the concentrations of 50 and 25% in their effect along the length of the plumule, reaching 4.5 and 3.8%, respectively. There was no significant difference between the interaction of both plant extracts and concentrations.

Figure (2) presents the summary statistics for the toxic effect of *M. phaseolina* on the length of the root. Figure (2) showed an encouraging effect for the growth of the plant root considering the extract of the *Cuminum cyminum* plant at 1.8 cm, which did not significantly differ than the *Zingiber officinale* extract, which gave a root length of 1.5 cm. *Cuminum cyminum* extract gave a significant effect on the length of the root which reached to 1.1 cm. There was no effect of the interaction between the concentrations 50 and 25% and the plant extracts on the length of the root, reaching 1.3 cm. There was also no significant effect between the examined concentrations of 50 and 25% on the length of the root, reaching 0.8 cm. This difference may be due to the differences between plant extracts and the difference between the active substances of each plant or to the type of active ingredient in each extract and thus negatively or positively affects the proportion of growth, plumules and the root [18].

Table 3. The toxic effect of *M. phaseolina* on the percentage of okra seeds in the dishes

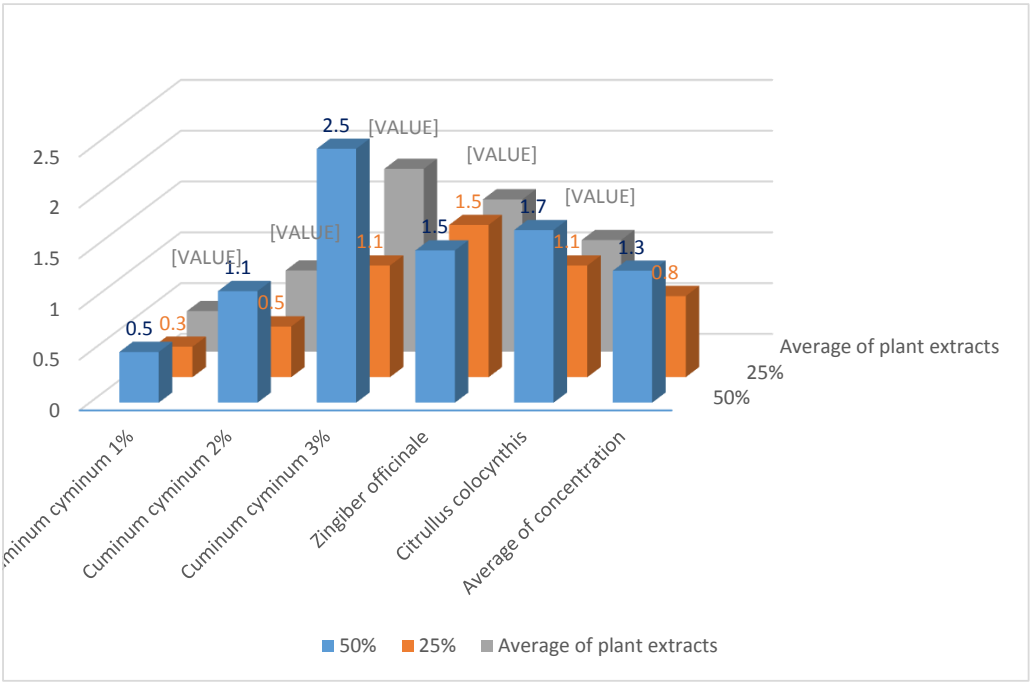
Plants Extract	Concentration		Average of plant extracts
	%50	25%	
Cuminum cyminum 1%	45	24.8	34.9
Cuminum cyminum 2%	45	36.6	40.8
Cuminum cyminum 3%	79.2	52.9	66
Zingiber officinale	59.1	47.6	53.3
Citrullus colocynthis	47.4	45	46.2
Average of concentration	55.1	41.4	

L.S.D. 0.05 for concentrations = 9.02 L.S.D. 0.05 for plant extracts = 7.8 L.S.D. 0.05 for interaction = 8.6



L.S.D. 0.05 for concentrations = N.S L.S.D. 0.05 for plant extracts = 1.9 L.S.D. 0.05 for interaction = N.S

Figure 1. The toxic effect of *M. phaseolina* on the length of plumule



L.S.D. 0.05 for concentrations = N.S L.S.D. 0.05 for plant extracts = 0.6 L.S.D. 0.05 for interaction = N.S

Figure 2. The toxic effect of *M. phaseolina* on the length of the root.

3.4 Effect of plant extracts in some of the characteristics of okra seedlings

Table (4) presents the effect of different concentrations of alcoholic plant extracts on the weight and root length of the okra. Closer inspection of the table shows an encouraging effect of *Cuminum cyminum* extract in seedling weight and root length. The seedling weight was 627.7 mg using the *Cuminum cyminum* extract, which differed significantly than the control treatment of 230 mg and the concentration of 3% gave the best increase of seeds weight which was 520.8 mg. There was a significant difference of the interaction between the concentrations and the type of plant extract. *Cuminum cyminum* extract showed a stimulated effect at concentration of 3% which was 670 mg with significant difference 230 mg.

As can be seen from Table (4), the *Cuminum cyminum* plant extract had a root length of 7.9 cm, which significantly differed than the control treatment of 2 cm. The concentration of 3% gave a root length of 6.6 cm and a significant difference than the other concentrations. The interaction between the extract type and the concentrations did not have any significant effect.

Table (5) shows the effect of different concentrations of alcoholic plant extracts on seedling length and percentage of okra seed germination. Table (5) showed the encouraging effect on seedling length of *Cuminum cyminum*, *Zingiber officinale* and *Citrullus colocynthis* extract, which were 10.9, 10.7 and 10 cm respectively, with significant differences than the control treatment, which reached 4.6 cm. The concentration of 3% was in the top of the concentrations in its effect on seedling, which showed a significant difference from the rest of the concentrations. The interaction between the extraction type and concentrations had no significant effect. The same table showed the promoting effect of the plant extracts on the percentage of growth, which reached 90% for all the studied extracts and a significant difference than the control treatment of 28.4%.

Several studies have indicated that *Cuminum cyminum* contain alcohols, tannins and alkaloids, as well as containing *Zingiber officinale* on alkaloids and phenols [19,11]. These compounds may stimulate systemic resistance and thus improve plant growth responses. Hammad et al [20] concluded to the important role of plant extracts because they contain active substances that promote the physiological processes of seeds such as breaking the dormancy phase and thus increasing the growth rate of seeds.

Table 4. The effect of different concentrations of alcoholic plant extracts on the weight and root length of the okra

Plant extracts	Seedlings weight (mg) Concentrations				Root length (cm) Concentrations			
	1%	2%	3%		1%	2%	3%	
Cuminum cyminum	575	636.67	670	627.7	7.1	7.5	9.2	7.9
Zingiber officinale	453.3	526.6	616	532.2	3.08	4.6	7.2	5.06
Citrullus colocynthis	346.6	443.3	566.6	452.2	2.5	5.5	8	5.3
Control	230	230	230	230	2	2	2	2
Concentrations average	401.2	459.1	520.8		3.6	4.9	6.6	

L.S.D. 0.05 of Seedling weight :

concentrations = 10.28 plant extracts = 9.52 interaction = 11.6

L.S.D. 0.05 of Root length :

concentrations = 1.2 plant extracts = 2.25 interaction = N.S

Table 5. Effect of different concentrations of alcoholic plant extracts on seedling length and percentage of okra seed germination

Plant extracts	Seedlings length (cm) Concentrations				% Percentage of okra seed germination Concentrations			
	1%	2%	3%		1%	2%	3%	
Cuminum cyminum	9	10.7	13	10.9	90	90	90	90
Zingiber officinale	9.1	10.6	12.5	10.7	90	90	90	90
Citrullus colocynthis	8	10.2	11.8	10	90	90	90	90
Control	4.6	4.6	4.6	4.6	28.4	28.4	28.4	28.4
Concentrations average	7.6	9.02	10.4		74.6	74.6	74.6	

L.S.D. 0.05 of Seedling length :

concentrations = 1.37 plant extracts = 4.3 interaction = N.S

L.S.D. 0.05 of % Percentage of okra seed germination :

concentrations = N.S plant extracts = 6.2 interaction = N.S

Conclusion

The main goal of the current study was to determine the efficiency of some plant extracts on the fungus *Macrophomina phaseolina*, the causes agent of seed rot and the Okra seedlings death. This study has shown that, *Cuminum cyminum* extract has a significant effect on *M. phaseolina*. These results add to the rapidly expanding field of the use of medicinal plants to reduce the effect of plant pathogens.

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