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Growth inhibitory effect of *Conocarpus lancifolius* plant aqueous extract on *Fusarium oxysporum* causal agent of wilt in some crops

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Abstract. *Elshair MASA, Mohamed IS. 2019. Growth inhibitory effect of* Conocarpus lancifolius *plant aqueous extract on* Fusarium oxysporum *causal agent of wilt in some crops. Cell Biol Dev 3: 81-85. Fusarium oxysporum* affects a wide variety of different ages tomatoes, tobacco, legumes, cucurbits, sweet potatoes, chickpea and Banana. The present investigation was undertaken to study the effect of Damas (*Conocarpus lancifolius* Engl.) plant parts (leaves, fruits, barks, and roots) aqueous extracts and fungicide Score (250 EC) on the growth of the fungus *F. oxysporum*, the causal agent of wilt disease in crops. Three concentrations of aqueous leaves, fruits, barks, and roots extract of *C. lancifolius*, each of 25, 50, and 100%, and fungicide was used in addition to control. The assessment of their inhibitory effect against the pathogen was recorded through the fungal growth. The results showed that all concentrations of the leaves, fruits, barks, and roots aqueous extracts *C. lancifolius* plant tested and fungicide showed significant inhibitory effect against the linear growth of *F. oxysporum* compared to control. Moreover, the concentration of each aqueous extract reacted differently against *F. oxysporum*. However, the highest concentration of the *C. lancifolius* extracts (100%) gave significantly higher inhibition zone percentages (75.5%, 68%, 66%, and 50%) than the untreated control. Among the *C. lancifolius* parts, extracts screened from the fruit (75.5) were more effective in suppressing the fungus growth than its equivalent other parts. The results showed that the antifungal activity increased with the extract concentration. The fungus *F. oxysporum* differs in its response to the different concentrations, but on the whole, growth inhibition increased with the concentration. The current results were considered promising and encouraging to carry out a phytochemical analysis of different parts of *C. lancifolius* plant using different solvents to determine the bioactive ingredient in each of these parts.

Keywords: *Conocarpus lancifolius*, damas plant, *Fusarium oxysporum*, growth inhibitory effect

INTRODUCTION

Fusarium oxysporum is a major cause of wilting (Nene et al. 1991). The disease is common in most tomato-producing countries and is a major disease. It is a disease transmitted by seeds and soil. The fungal pathogen *F. oxysporum* affects a wide variety of hosts of different ages tomatoes, tobacco, legumes, cucurbits, sweet potatoes, chickpeas, and bananas are among the most susceptible crops, but other herbaceous plants are also affected (Pan Germany 2010).

Fusarium wilt is a common causative agent of vascular wilt, which has similar symptoms to verticillium wilt. The causative agent of *Fusarium* wilt is *F. oxysporum* (Snyder and Hansen 1940). The species is further divided into forma specialists based on the host plant. These fungi typically produce symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of the vascular system, stunting, and damping. The most important of these are vascular wilt. *F. oxysporum* is a common soil saprophyte and pathogen that feeds on dead and decaying organic matter. It survives in soil debris such as mycelium and all types of spores but is often recovered from the soil as chlamydospores (Snyder and Hansen 1940). It is a major wilt pathogen of many economically important crops. It is a soil-borne pathogen that can live in the soil for a long time, so rotational cultivation is not a useful control method. It can also spread through infected dead plant matter, so cleaning up late in the season is important. Members of *F. oxysporum* are found all over the world.

Before global transport, however, many of the different pathogen varieties had been isolated. Now world trade has spread *F. oxysporum* inoculums with the crop. A recent example of this is the spread of *F. oxysporum* f.sp. Cubense, may have originated in Asia and recently appeared in banana-growing areas in the South Pacific (Davis and Richard 2004). In Sudan, various diseases are known to limit agricultural production. One is *Fusarium* wilt caused by *F. oxysporum*, a major disease-causing economic loss (Bhatia et al. 2004). The disease is particularly severe in traditional production areas. Based on the above, this study was conducted to focus on research on two components for managing *Fusarium* wilt caused by *F. oxysporum*, superior plant extracts, and synthetic fungicides under laboratory conditions to formulate a promising disease management approach. With the following objectives: (i) To study the antifungal potential of some higher plants, crude extract against *F. oxysporum*. (ii) Evaluate the effect of the systemic fungicide on fungal growth. (iii) Development of promising components for *Fusarium* wilt disease management.

MATERIALS AND METHODS

This study was conducted under laboratory conditions at the Department of Plant Pathology, College of Agricultural Studies "Shambat," Sudan University of Science and Technology (SUST), Sudan, from November 2015 to February 2016 to evaluate the inhibitory effect of all parts of damas (*Conocarpus lancifolius* Engl.) (leaves, bark, fruit, and root) aqueous extracts and fungicidal efficacy, Score 250 EC, against the fungus *F. oxysporum.*

Collections of plant samples

Various parts of *C. lancifolius* (fruits, leaves, bark, and roots) were collected from trees growing in the Elshair Farm Project. Collected parts were cleaned of dust and foreign matter by hand, washed with distilled water, surface sterilized with 1% sodium chloride, washed thoroughly in sterilized water, and dried in the shade at room temperature, ground, and pulverized separately to obtain a fine powder for extraction and is preserved for use.

Preparations

Preparation of plant extract

All parts of *C. lancifolius* (leaves, bark, fruit, roots) were collected from the elshair farm project and dried in the shade. After the plants were completely dry, the plants were ground separately to obtain fine powder for extraction.

Preparation of inoculums

Pure cultures of *F. oxysporum* were prepared using 7-day-old mycelia. The fungi were grown on PDAs and then aseptically transferred to the center of Petri dishes containing PDA medium and incubated at 25°C. Linear growth of the fungus was determined in cm after 72 hours.

Aqueous extract preparation

Aqueous extracts of each plant material were prepared as recommended by (Okigbo and Ogbonnaya 2006). The fine powder obtained from various parts of *C. lancifolius* was weighed (100 g), and 100 mL of sterilized distillate was added to it in a 250 mL Erlenmeyer flask and then placed in a shaker for 24 hours. The extracts were filtered under reduced pressure as 100% raw water extract. The other concentrations obtained were diluted to 50% and then 25% and stored in the refrigerator to serve as stock solutions.

Preparation of fungicide

The fungicide tested was Score of which 2 mL were dissolved in 1000 mL of sterilized distilled water to give 100 ppm.

The effect of each extract was calculated as the percentage of reduction in diameter of fungal growth (R) where:

$$R = \frac{dc - dt}{dc} \times 100$$

Where R = Percentage reduction of the growth, dc = diameter of controlled growth, and dt = diameter of treated growth

Effect of different parts of *C. lancifolius* extract on the linear growth of the *F. oxysporum*

The PDA medium was supplemented with the required concentration of all parts of *C. lancifolius* and Fungicide Evaluation (25 mL, 50, and 100 mL each) before being solidified in a 250 mL Erlenmeyer flask, stirred, and poured into Petri dishes Sterilized. Three panels were assigned to each concentration and allowed to solidify. The other three plates of PDA medium served as controls. Petri dishes of each concentration were incubated at 25°C for 5 days. The growth diameter of the fungus was measured and calculated in centimeters 3, 4, and 5 days after inoculation.

Experimental design

The treatments were arranged in a Complete Randomized Block Design.

Statistical analyses

The obtained data were statistically analyzed according to the analysis of variance (ANOVA); Duncan's Multiple Range Test was used for mean separation.

RESULTS AND DISCUSSION

This study was conducted under laboratory conditions by Jica, College of Agricultural Studies, Sudan University of Science and Technology, Sudan, between November 2015 and February 2016 to investigate the inhibitory effect of all parts of aqueous extracts of *C. lancifolius* (leaves, bark, fruits, roots) and fungicide, achieving an efficacy of 250 EC against the growth of the fungus *F. oxysporum.*

Isolation and Identification from the infected sample

The isolation and identification of the fungus were based on the method of (Booth 1977) and on the colony's characteristics and microscopic examination. In addition, standard books and research papers were consulted in the search for these mushrooms (Aneja 2004).

Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after three days from inoculation

The results (Table 1, Table 2, and Figure 1) showed that all aqueous extracts of *C. lancifolius* (leaves, fruits, barks, roots) were screened, and the fungicidal effects on fungal growth had three days after inoculation. In addition, fungal growth inhibition was significantly elevated compared to the control.

Additionally, the highest concentration of plant extracts (100%) gave significantly higher inhibition than the untreated control, which gave (75.5%, 68%, 66%, and 50%). Of the tested parts of *C. lancifolius* extracts, the fruit was more effective in suppressing fungal growth than other parts of *C. lancifolius*, each producing (75.5) in (Table 1, Table 2), the results showed that the concentration of antifungal activity increases with increasing extract.

Table 1. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after three days from inoculation

Treat.	Conc.	Growth			
		R1	R2	R3	Mean
Leaves	25	4(2.1)	4.1(2.1)	4(2.1)	4.033(2.1)ab
	50	3.9(2.1)	4(2.1)	3.9(2.1)	3.93(2.1)ab
	100	2.5(1.7)	2.7(1.8)	3.1(1.9)	2.76(1.8)bc
Fruit	25	4(2.1)	4.1(2.1)	3.9(2.09)	4(2.09)ab
	50	2.05(1.6)	1.9(1.5)	2.2(1.6)	2.05(1.6(cd
	100	1.4(1.4)	1.2(1.3)	1.6(1.4)	1.4(1.36)d
Bark	25	3.5(2)	3.7(2)	3.3(1.7)	3.5(1.9)bc
	50	3(1.9)	2.5(1.7)	2.8(2)	2.76(1.8)bc
	100	1.6(1.4)	2(1.6)	1.7(1.4)	1.76(1.46)d
Root	25	3(1.9)	3.5(2)	3.7(2)	3.4(1.96)abc
	50	2.2(1.6)	2.6(1.8)	2.1(1.6)	2.3(1.66)cd
	100	1.7(1.4)	2.3(1.8)	2(1.6)	2(1.6)cd
Fungicide		0.3(0.9)	0(0.7)	0.4(0.7)	0.233(0.76)e
Control		5(2.3)	4.9(2.3)	5(2.3)	4.96(2.3)a
C.V				11.74	
SE				0.06	

Note: Means in the same column with the same letter (s) are not significant at P=0.05, according to DRMT. Values between brackets were transformed to √x+0.5

Table 2. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after five days from inoculation

Treatments	Conc.	Inhibition zone			
		R1	R2	R3	Mean
Leave	25	20.00	16.30	20.00	18.30
	50	22.00	18.30	22.00	20.70
	100	50.00	44.00	38.00	44.00
Fruit	25	20.00	16.30	22.00	19.40
	50	59.00	61.20	56.00	58.70
	100	72.00	75.50	68.00	71.80
Bark	25	30.00	24.40	34.00	29.50
	50	40.00	48.90	44.00	44.30
	100	68.00	59.18	66.00	64.40
Root	25	40.00	28.50	26.00	31.50
	50	56.00	49.00	58.00	54.30
	100	60.00	59.18	66.00	61.10
Fungicide		94.00	100.0	92.00	95.30
Control		00.00	00.00	00.00	00.00

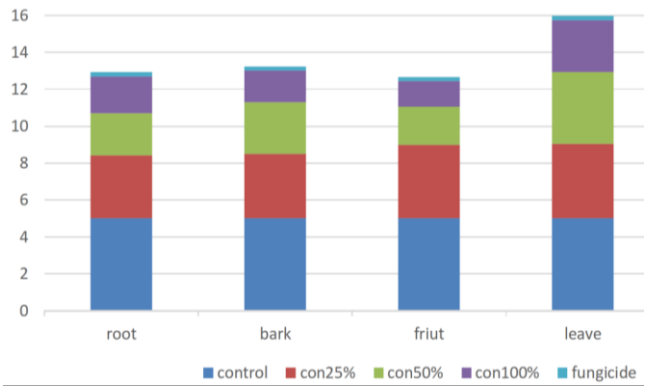

Figure 1. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after three days from inoculation

Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after four days from inoculation

Four days after inoculation, all parts of *C. lancifolius* plants tested concentrations, as well as that of the fungicide, invariably continued and showed inhibitory effects against fungal growth. However, the highest concentration of plant extracts (100%) gave the highest percentage of inhibition zones (68.57%, 42.85%, 57.14, and 55.71, respectively). This inhibitory effect of all concentrations tested significantly differed from the control (Table 3, Table 4, and Figure 2). Furthermore, the fruit part of the *C. lancifolius* plant extract remained the most suppressive at all tested concentrations, followed in descending order by the other parts.

Effect of different parts of *C. lancifolius* plant aqueous extracts and 4 on the linear growth of *F. oxysporum* after five days from inoculation

Within five days of inoculation, the results (Table 5, 6, and Figure 3) showed that extracts from all tested parts of *C. lancifolius* plants effectively suppress fungal growth. Indeed, all concentrations of all parts of *C. lancifolius* tested (100, 50, and 25%) induced significantly greater inhibition against the test fungus than the control, which gave (63.75, 43.75.59, 37, and 58.12%). Meanwhile, the aqueous fruit extract in high concentrations tested consistently showed a greater inhibitory effect than the other parts of the *C. lancifolius* aqueous plant extracts that give (63.75). It is clear that the organism under examination differs in its response to different concentrations of plant extracts, but in general, growth inhibition increases with concentration. This inhibitory effect of all concentrations was significantly different from the control.

Table 3. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after four days from inoculation

Treat.	Conc.	Growth			
		R1	R2	R3	Mean
Leave	25	5.75(2.5)	5.9(2.6)	5.75(2.5)	5.8(2.5)ab
	50	5.25(2.4)	5.7(2.5)	5.25(2.4)	5.4(2.4)bc
	100	3.75(2.1)	4(2.1)	4.1(2.1)	3.95(2.1)of
Fruit	25	4.5(2.2)	4.35(2.2)	4.5(2.2)	4.45(2.2)de
	50	2.9(1.8)	2.5(1.7)	2.9(1.8)	2.8(1.8)gh
	100	2.4(1.7)	2.2(1.6)	2.3(1.6)	2.3(1.63)h
Bark	25	5.8(2.5)	5.6(2.4)	5(2.3)	5.46(2.4)bc
	50	4.6(2.3)	4(2.1)	4.7(2.5)	4.43(2.3)cd
	100	3.0(1.9)	3.5(2)	3.3(1.9)	3.26(1.9)fg
Root	25	5.5(2.4)	5.7(2.5)	5.45(2.3)	5.6(2.4)bc
	50	3.5(2)	3.8(2)	3.9(2.1)	3.7(2.0)of
	100	3.1(1.9)	3.5(2)	3.5(2)	3.4(1.9)f
Fungicide		0.9(1.1)	0(0.7)	0.8(1.1)	0.56(0.96)i
Control		7.0(2.7)	7.0(2.7)	7.0(2.7)	7.0(2.7)a
C.V				4.71	
SE				0.07	

Note: Means in the same column with the same letter (s) are not significant at P=0.05, according to DRMT. Values between brackets were transformed to √x+0.5

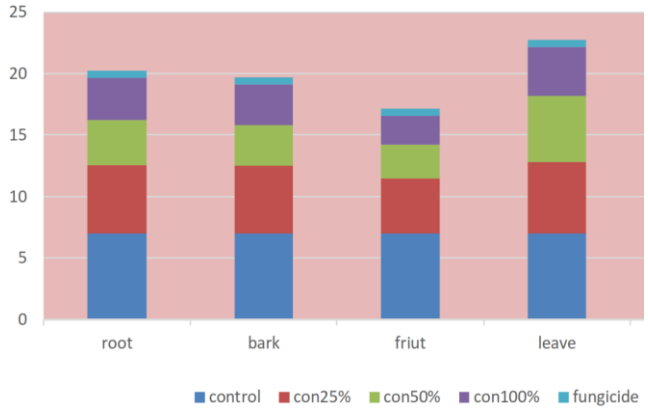

Figure 2. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after four days from inoculation

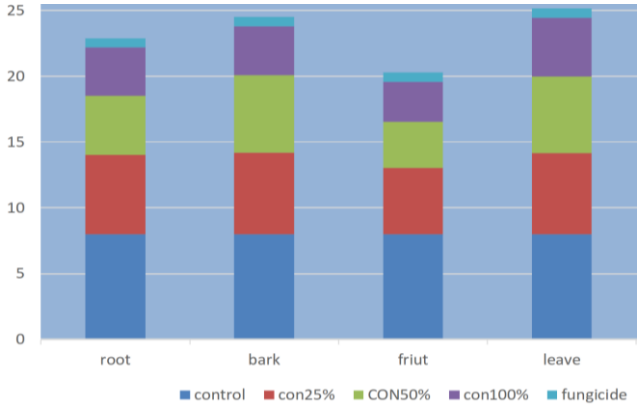

Figure 3. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after five days from inoculation

Table 4. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after four days from inoculation

Treatments	Cons.	Inhibition zone			
		R1	R2	R3	Mean
Leave	25	17.85	15.71	17.85	17.14
	50	25.00	18.5	25.00	22.83
	100	46.42	42.85	41.42	43.56
Fruit	25	35.7	37.8	35.7	36.40
	50	58.5	64.28	58.57	60.45
	100	65.71	68.57	68.24	67.50
Bark	25	17.14	20.00	28.57	21.90
	50	34.28	42.85	32.85	36.66
	100	57.14	50.00	52.28	53.14
Root	25	21.42	18.57	22.41	20.80
	50	50.00	45.71	44.28	46.66
	100	55.71	50.00	50.00	51.90
Fungicide		87.14	100.0	88.57	91.90
Control		00.00	00.00	00.00	00.00

Table 5. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after five days from inoculation

Treat.	Cons.	Growth			
		R1	R2	R3	R4
Leaves	25	6.15(2.6)	6.1(2.6)	6.2(2.6)	6.15(2.6)b
	50	5.56(2.5)	5.9(2.5)	5.8(2.5)	5.8(2.5)bc
	100	4.35(2.2)	4.5(2.2)	4.7(2.3)	4.51(2.2)d
Fruit	25	4.9(2.3)	4.9(2.3)	5.3(2.4)	5.0(2.3)cd
	50	3.7(2.05)	3.5(2)	3.35(2)	3.5(2.02)cd
	100	3(1.87)	2.9(1.8)	3.3(1.9)	3.06(1.85)f
Bark	25	6.65(2.6)	6.1(2.6)	6.4(2.6)	6.21(2.6)b
	50	6(2.5)	6(2.5)	5.7(2.5)	5.9(2.5)bc
	100	3.35(1.9)	4(2.1)	3.9(2.09)	3.71(2.03)of
Root	25	5.7(2.5)	6.25(2.6)	6.1(2.6)	6.016(2.56)b
	50	3.9(2.1)	4.5(2.2)	5(2.3)	4.46(2.2)de
	100	3.35(2)	4(2.1)	3.7(2)	3.68(2.03)ef
Fungicide		1.4(1.4)	0(0.7	0.8(1.1)	0.73(1.06)g
Control		8(2.9)	8(2.9)	8(2.9)	8(2.9)a

Note: Means in the same column with the same letter (s) are not significant at P=0.05, according to DRMT. Values between brackets were transformed to √x+0.5

Table 6. Effect of different parts of *C. lancifolius* plant aqueous extracts and fungicide on the linear growth of *F. oxysporum* after four days from inoculation

Treatments	Cons.	Inhibition zone			
		R1	R2	R3	Mean
Leave	25	23.12	23.75	22.50	23.12
	50	29.37	26.25	27.50	27.70
	100	45.62	43.75	41.25	43.54
Fruit	25	38.75	38.75	33.75	37.08
	50	53.75	56.25	58.12	56.04
	100	62.50	63.75	58.12	61.45
Bark	25	33.25	23.75	20.00	25.66
	50	25.00	25.00	28.75	26.25
	100	59.37	50.00	51.25	53.54
Root	25	28.75	21.87	23.75	24.79
	50	51.25	43.75	37.50	44.16
	100	58.12	50.00	53.75	53.95
Fungicide		82.50	100.0	90.00	90.83
Control		0.000	0.000	0.000	00.00

Discussion

The major pathogens of *Fusarium* wilt have a wide range of host plants and include numerous special forms, some contain two or more pathogenic breeds, causing devastating wilt diseases, and many are carried by seeds as listed by (Andersen 1974) for the following host's Alliums cannabis, Beta vulgaris, Cucumis sativa, Phaseolus vulgaris, and Psumist sativum.

The *F. oxysporum* is a major cause of wilting (Nene et al. 1991). The disease is common in most tomato-producing countries and is a major disease. It is a disease of seeds and soil. The fungal pathogen *F. oxysporum* affects a variety of hosts of different ages, such as tomatoes, tobacco, legumes, and cucurbits. Sweet potatoes, chickpeas, and bananas are among the most susceptible crops, but other herbaceous plants are also affected (Pan Germany 2010).

Several diseases are known to limit crop yields in Sudan. One of them is *Fusarium* wilt caused by *F. oxysporum*, which is one of the most important diseases causing economic losses (Bhatia et al. 2004). The disease is

considered especially severe in traditional production areas where crops are grown on stored soil moisture after the Nile floodwaters have subsided. As a result, farmers do not adhere to crop rotation in these areas, and the crop in the post-flowering phase is often subject to water stress during low tide years (Ali 1996).

A large body of research has identified various strategies to combat this fungal pathogen (Haware and Nene 1982). However, treating seed- and soil-borne diseases such as wilt caused by *F. oxysporum* has always been problematic (Haware and Kannaiyan 1992; Rao and Balachadran 2002). In general, synthetic fungicides greatly reduce the incidence of wilt, but their use is expensive and harmful to the environment (Song and Goodman 2001). Moreover, the use of resistant cultivars is fraught with resistance breakdown due to high pathogen variability in the pathogen population (Kutama et al. 2011). In this context, searching for an ecological way to control *Fusarium* wilt in crops that offer an alternative to fungicides is very challenging.

Historically, numerous phytochemicals isolated from various plants are now prescribed by physicians worldwide (Newman et al. 2000). Many plant extracts or products are as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand, botanical insecticides have great advantages over synthetic pesticides because they are more environmentally friendly and are accepted by most farmers, government organizations, and decision-makers.

In this study, the different parts of the aqueous extracts of the *C. lancifolius* plant were examined for their biological activity against *Fusarium* wilt. The data (Tables 1-3 and Figures 1-3) revealed that all screened aqueous extracts of *C. lancifolius* plants (leaves, fruits, bark, and root) consistently showed an inhibitory effect on fungal growth with a significantly high percentage of zones of inhibition. This result is in agreement with Satish et al. (1999), Ergene et al. (2006), Kiran and Raveesha (2006), Mohana and Raveesha (2006), Okigbo and Ogbonnaya (2006), and Sharif et al. (2006); who studied the effects of extracts from many higher plants and reported that they had antibacterial, antifungal and insecticidal properties in laboratory tests. More recent results have also been published by Saad et al. (2014), where they demonstrated the antibacterial and antifungal activities of the methanol extract of *C. lancifolius* air pieces using the disk diffusion method. Similar results were also obtained by Ahmed (2014), who used the alkaloid extract of *C. lancifolius* against certain clinical pathogens. From this study, it can be concluded that: (i) the extracts of leaves, fruits, bark, and roots of the *C. lancifolius* plant tested showed an inhibitory effect on the growth of fungi. *F. oxysporum* This more fungicidal component (score) could be applied as part of an integrated approach to control *Fusarium* wilt. (ii) Of the plant parts of *C. lancifolius*, the aqueous extract in high concentration showed an inhibitory effect compared to the others. (iii) The screened concentrations of all parts of *C. lancifolius* aqueous extracts differ in their responses to fungal assays. Likewise, the test organism reacted differently to different concentrations of extracts. This variability in the response expressed by the test organism can be used to adjust an optimal dose to control *Fusarium* wilt.

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