In vitro antifungal activity of Non Pathogenic fungi against the Fusarium spp. causing rot disease on Aloe vera.

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ABSTRACT: *The present in vitro study was carried out to access the efficacy of fungi (non pathogenic) against Fusarium* spp.*responsible for causing rot disease on Aloe vera leaves. The four non pathogenic fungi viz Rhizoctonia sp., Rhizopus nigricans, Trichoderma sp., Aspergillus fumigatus, isolated from healthy leaves of Aloe vera were used for antagonistic activity. Two methods Dual culture and Cultural filterate technique were used for testing antagonistic activity against the pathogenic Fusarium* spp.*, results revealed that Rhizopus nigricans., Trichoderma sp. and Aspergillus fumigatus showed maximum inhibitory effect on rot disease causing Fusarium* spp.*Thus they can be utilized as biological agent to control Fusarium* spp. *responsible for causing rot disease in Aloe vera.*

Key Words: *Aloe vera, leaf rot, Pathogenic Fungi, Non pathogenic Fungi, Fusarium* spp.

Introduction

Aloe Vera (*Aloe barbadensis* Miller) is an important medicinal plant known for its medicinal values. *Aloe* is derived from the Arabic word "Alloeh" which means "shin-ning bitter substances" (Akinyele and Odiyi, 2007). Among several species of Aloe were found, the common varieties are: *Aloe barbadensis Mill, Aloe saponaria, Aloe chinensis, Aloe variegata, Aloe forex, Aloe lalifolia* and *Aloe curacao* but *Aloe vera* (Syn. *Aloe barbadensis* Miller) is more popular all over the world because it propagates itself faster than any other known species. Hence *A. vera* is more readily available for use than any other species of *Aloe.* This plant having more therapeutic value and referred as 'True Aloe' and also known as a richest sources of health for human beings which is coming from nature (Shukla et al., 2008). It is in high demand in pharmaceutical industries and also important for villagers, as they used this plant as a medicine because it is important for curing many diseases this plant is also known as miracle plant.

It is observed that every year crop is been damaged by various diseases and among them fungal diseases are very common, (Hewitt, 2000). Severe spotting on leaves was reported which is most dangerous, especially for villagers because they consumed *Aloe* as a medicine. For the prevention of leaf rot disease various synthetic fungicides are used, which is very common and gives positive results as well, but several side-effects was also noticed in the form of carcinogenicity, detrimental effects and other residual toxicities. Because of such side-effects there is a necessity to find an alternative choice which is potentially active. Fungicides of plant origin known as botanical fungicides are used which are specific biodegeradable, non-phytotoxic, cheap, readily available and environmentally safe than synthetic chemicals with fewer side effects (Okigbo and Ogbonna, 2006; Abad et al., 2007 ; Yazdani et al., 2011).Antimicrobial activity of native fungus were investigated and also check which one is potentially effective with variable efficiency against the pathogen. During the study several pathogens were observed but *Fusarium equiseti, Fusarium beomiforme, Fusarium Fujikuroi, Fusarium circinatum* ,were highly pathogenic (data not shown). Various fungus isolated from healthy *Aloe vera* leaves were screened for antagonistic activity against Pathogenic Fusarium spp.

Material and Method

Survey and Collection

The *Aloe vera* leaves were collected from Jabalpur region during the year 2015, every month, both, healthy and infected leaves (leaves having visible black spots) were collected for studies, leaf samples were cut off with an ethanol-disinfected knife and placed separately in sterile polythene bags and stored in an icebox, collected samples were used to isolate the fungi within 48 h of collection.

Isolation and Identification of fungi from *Aloe vera* leaves

In order to get the mycoflora (the fungi) on *Aloe vera* leaves, both healthy and infected leaves, were cultured. For this, the leaf samples were cut into small pieces of 1 mm^2. These small pieces were used to isolate different fungi present on the leaf of *Aloe vera*. Different isolation methods were used to culture the mycoflora (data not shown). These isolated fungi were identified using cultural and microscopic characters using standard literature. Molecular identification is also being used for selected fungi. After testing pathogenecity of fungus (data not shown) isolated from infected *Aloe vera* leaves on *Aloe vera*, potent one is used for further studies, and fungi isolated from healthy *Aloe vera* leaves will be taken for antagonistic activity against the pathogenic fungi.

Antagonistic activities of leaf surface mycoflora against Fusarium spp.

Dual culture method

The antagonistic potential of different fungal isolates from healthy leaves of *Aloe vera* were evaluated against different isolates of *Fusarium.* The dual culture technique as specified by Sharfuddin and Mohanka (2012) was used.

Mycelial disc of each test antagonist taken from 7 day old culture was paired against same sized mycelia disc of *Fusarium* sp. isolated during the study at opposite end on PDA (20 ml) contained in 90 mm diameter Petri- plates. The pathogen and antagonist disc were place at equal distances from the periphery of the petriplate. The PDA plates inoculated only with phytopathogen served as control. The plates were incubated at 28 ± 2°C. The growth of the pathogen in both test and control experiments were recorded and the percent inhibition of radial growth was calculated as-

$$\% \text{ inhibition} = (R_1 - R_2)/R_1 \times 100.$$

Where

R_1 = radial growth of pathogen in control.

R_2 = radial growth of pathogen with antagonists.

Culture filtrate method

The fungal isolates from healthy *Aloe vera* leaves showing antagonistic activities in dual culture method were further verified by the culture filtrate method. For this, the inhibition of the mycelial growth of plant pathogen was tested by metabolites secreted by fungal isolates in liquid medium.

The sterilized filtrate were amended in PDA in four concentrations (250 µl, 500 µl, 750 µl and 1000 µl) in Petri plates. The mycelial discs of the pathogen were placed at the centre of solidified agar plates and incubated at optimum temperature for 7 days. Plates devoid of culture filtrates served as control. Radial growth of *Fusarium spp.* isolated during the study was measured and its inhibition percentage of mycelia growth was calculated using the formula:

$$I = [(C_2 - C_1)/C_2)] \times 100$$

I = percentage inhibition of radial mycelial growth,

C_2 is radial growth of pathogen in control.

C_1 is radial growth of pathogen in treated plates.

Result and Discussion

Antagonistic effect of Fungi isolated from healthy *Aloe vera* leaves

The fungi isolated from the healthy *Aloe vera* leaves during the present study were analyzed for their ability to counter the growth, and hence pathogenicity of isolated *Fusarium* species. The antagonistic activity was shown firstly using dual culture method where the isolated Fusarium species were co-cultured with fungi isolated from healthy *Aloe vera* leaves. The suppression of radial mycelia growth of Fusarium species with other fungi was the indicator of antagonistic activity, and the percent inhibition of radial growth was calculated with the radial growth when the Fusarium species were allowed to grow alone.

Table 1 shows the percent inhibition of radial mycelia growth of Pathogenic Fusarium spp. when co-cultured with non pathogenic fungi.

When *Fusarium fujikuroi*, *Fusarium circinatum* and *Fusarium beomiforme* was co-cultured individually with other isolated fungi. Maximum growth inhibition was observed by *Rhizopus nigricans* i.e 67.95%, 73.26% and 73.03% respectively.

Similarly when *Fusarium equiseti* when co-cultured with other isolated fungi. Maximum growth inhibition was observed by *Trichoderma sp.* i.e. 75.58% respectively.

In second phase of experiments for antagonistic activities by the healthy leaf mycoflora against pathogenic Fusarium species was done using the culture filtrate method in a dose dependent manner. The culture filtrate of 15 days old healthy leaf mycoflora was added to the culture medium (PDA) in the

concentration of 250 µl, 500 µl, 750 µl and 1000 µl, and the Fusarium species were allowed to grow in these medium. Control received no culture filtrate.

Table 2 shows the suppression of radial mycelia growth of all four Fusarium spp. *F. equiseti, F.circinatum, F.beomiforme, F.Fujikuroi* with culture filtrates of non pathogenic fungi in four concentrations. Maximum growth inhibition was observed by *Aspergillus fumigatus.*

Conclusion

Aloe Vera (*Aloe barbadensis* Miller) is miracle plant known for its medicinal values, more popular all over the world because it propagates itself faster also more readily available for use than any other species of *Aloe.* Severe spotting on leaves was reported which is most dangerous, especially for villagers because they consumed *Aloe* as a medicine. For the prevention of leaf rot disease various synthetic fungicides are used, which have several side-effects and controlling disease by biocontrol measure especially fungi is more promising alternative choice. Thus in the present study several pathogens were isolated from diseased *Aloe vera* among which *Fusarium equiseti, Fusarium beomiforme, Fusarium Fujikuroi, Fusarium circinatum* ,were found highly pathogenic. Similarly various fungus were also isolated from healthy *Aloe vera* leaves and were screened for antagonistic activity against these pathogens, it was found that among all four antagonistic fungi *Rhizopus nigricans, Trichoderma* sp.*, Aspergillus fumigatus* showed the maximum antagonistic activity. These isolated fungi proved to be the potent biocontrol agent against all the pathogenic *Fusarium* isolates.

Table 1: Effect of antagonists on mycelial growth and per cent growth inhibition of *Fusarium* spp.

S.no.	Fusarium spp.	Antagonistic fungi	Zone Size in mm 7th day	% inhibition after 7th day
1	*F. fujikuroi*	Control	78	
		Rhizoctonia sp.	26	66.67
		Rhizopus nigricans	25	67.95
		Trichoderma sp.	27	65.38
		Aspergillus fumigates	26	66.67
2	*F. circinatum*	Control	86	
		Rhizoctonia sp.	24	72.09
		Rhizopus nigricans	23	73.26
		Trichoderma sp.	26	69.77
		Aspergillus fumigates	24	72.09
3	*F. beomiforme*	Control	89	
		Rhizoctonia sp.	25	71.91
		Rhizopus nigricans	24	73.03
		Trichoderma sp.	26	70.79
		Aspergillus fumigates	26	70.79
4	*F. equiseti*	Control	86	
		Rhizoctonia sp.	26	69.77
		Rhizopus nigricans	24	72.09
		Trichoderma sp.	21	75.58
		Aspergillus fumigates	22	74.42

Table .2: Effect of culture filtrate of antagonists on per cent growth inhibition of *Fusarium* spp. in vitro.

S. no.	Fusarium isolates	Antagonistic fungi	250 µL		500 µL		750 µL		1000 µL	
			growth (mm)	% inhibition	growth (mm)	% inhibition	growth (mm)	% inhibition	growth (mm)	% inhibition
1	*F. equiseti*	Control	87	-	87	-	87	-	87	-
		Rhizoctonia sp.	59	32.18	55	36.78	48	44.83	46	47.13
		Rhizopus nigricans	64	26.44	58	33.33	54	37.93	48	44.83
		Trichoderma sp.	72	17.24	65	25.29	56	35.63	42	51.72
		Aspergillus fumigatus	64	26.44	52	40.23	43	50.57	28	67.82
2	*F. beomiforme*	Control	86	-	86	-	86	-	86	-
		Rhizoctonia sp.	61	29.07	57	33.72	49	43.02	48	44.19
		Rhizopus nigricans	65	24.42	60	30.23	53	38.37	50	41.86
		Trichoderma sp.	74	13.95	68	20.93	55	36.05	44	48.84
		Aspergillus fumigatus	66	23.26	54	37.21	45	47.67	30	65.12
3	*F. fujikuroi*	Control	84	-	84	-	84	-	84	-
		Rhizoctonia sp.	62	26.19	56	33.3	46	45.2	42	50
		Rhizopus nigricans	64	23.81	58	31	49	41.7	45	46.4
		Trichoderma sp.	73	13.1	66	21.4	52	38.1	48	42.9
		Aspergillus fumigatus	64	23.81	55	34.5	46	45.2	32	61.9
4	*F.circinatum*	Control	88	-	88	-	88	-	88	-
		Rhizoctonia sp.	63	28.41	56	36.4	42	52.3	38	56.8
		Rhizopus nigricans	62	29.55	55	37.5	47	46.6	42	52.3
		Trichoderma sp.	71	19.32	63	28.4	52	40.9	48	45.5
		Aspergillus fumigatus	62	29.55	57	35.2	45	48.9	33	62.5

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