Annals of Plant and Soil Research 15(2): 97-100 (2013)

ANTI-FUNGAL EVALUATION OF *ALOE VERA LEAF EXTRACT AGAINST* SOME PLANT PATHOGENIC FUNGI

RAJNI SINGH, SASODE D.S. SASODE AND P.K. JAGA*

Department of Plant Pathology, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, College of Agriculture Gwalior (M.P.) - 474 002
Received: Febrauary 2013, Revised accepted: November 2013

ABSTRACT

The present experiment was conducted during 2010-12 to screen three forms of Aloe vera leaf extracts viz., crude (20%), powder (20%), and ethanol (1%) for antifungal activity against ten test pathogens viz Rhizoctonia solani, Fusarium oxysporum f.sp. ciceri, Sclerotium rolfsii, Phoma sorghina, Colletotrichum gloesporioides, Alternaria solani, Alternaria alternata, Tolyposporium penicillariae, R. bataticola, and Alternaria brassicae at College of Agriculture, Gwalior. All the forms of Aloevera extract significantly inhibited the growth of the test fungus. The antifungal activity of crude extract (20%) was more effective than ethanol and powder extracts against eight fungal pathogens viz. R. solani, Fusarium oxysporium f.sp.ciceri, Sclerotium rolfsii, C.gloesporioides, A.solani, T.penicilarium, R.bataticola, A.brassicae. The ethanol extract of Aloe vera leaf extract was more effective against two fungal pathogen viz. Phoma sorghina, A.alternata. It was observed that the effectivity of crude and ethanol extract against the respective pathogenic fungi was gradually increased with the increase in the concentration from 20 to 50%, and 1 to 4% respectively on comparison with carbendazim (0.1%) and mancozeb (0.2%), it was observed that both the chemicals were found more effective than the Aloevera leaf extract (crude/powder/ethanol). Therefore, higher concentration of different forms of Aloevera leaf extract should be tested under in-vitro condition and the effective one may also be tested in the field as an alternative to the chemical for the eco-friendly management of the disease.

Key words: Leaf, crude, powdered and ethanol extracts, test pathogen, chemicals, antifungal activity.

INDRODUCTION

Aloe vera popularly known as Ghee-kunvar; Ghikumari, is a xerophytic perennial herb with fleshy leaves, which has a great therapeutic value as well as cosmetic importance (*Eggli,* 2001). The plants belong to family *Liliaceae* which has numerous species. *Aloe vera Barbadensis* Miller is the only plant that is known to have legendary medicinal reputation dating back to thousands of years ago (Anonymous, 2006). *Aloe vera* plant products have long been used for its medicinal purposes (Haller, 1990; Grindly and Reynolds, 1986]. Though, *Aloe vera* is more useful for healing but it is also used for its antifungal and antibacterial properties. *Aloe vera* plant possesses six antiseptic agents, tupoel, salicylic acid, urea, nitrogen, cinnamon acid, phenol and sulphate. Its antifungal quality is emphasized in many fields of medicines. The leaf pulp of *Aloe vera* designated as the gel and the bitter yellow liquid fraction have been tested against pathogens (bacteria and fungi) affecting human and plants. In spite of tremendous quality of the plant, its activity against plant pathogen in commercial industrial crops has not been determined. Therefore, botanicals are gaining importance in crop protection in view of their selective properties, low cost and safety to ecosystem. Many botanicals have been identified to be effective in the control of plant diseases. Among the 5280 species tested, 1134, 346 and 92 plant species possessed insecticidal, fungicidal and bactericidal properties, respectively (Ahmed and Grainge, 1982). The objective of this study is to evaluate the inhibitory effect of *Aloe vera* extract on the mycelial growth of different phytopathogenic fungi and to determine the effective concentration tha can inhibit mycelial development.

MATERIALS AND METHODS

The experiment was conducted at College of Agriculture, Gwalior during 2010-11 and 2011-12. The poisoned food technique was used to test antifungal activity of Aloe vera extract against ten plant pathogenic fungi *viz*., *Rhizoctonia solani, Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii, Phoma sorghina, Colletotrichum gloesporioides, Alternaria solani, Alternaria alternata, Tolyposporium penicillariae, R. bataticola,* and

JNKUV, College of Agriculture, Ganj basoda, Vidisha (MP) 464 221
Corresponding author Email: rnikum_ujjain@yahoo.co.in

Alternaria brassicae the pathogens were isolated from infected host and the selection of media is based on the standard recommendation for culturing of these fungi. Various extracts of Aloevera were prepared.

Powdered extract: Thoroughly washed fresh plant leaves were oven dried at 60^0C for 15 consecutive days. After drying the leaves were crushed by mixer. The powder of crushed leaves was stored in the airtight plastic bottles. The powder was used at the concentration of 20 % for this 20 gm powder was incorporated into 100 ml of distilled water then it was kept for 24 hours. Thereafter, it was filtered into a measuring cylinder and 500 ml volume of the extract was maintained by adding the water and finally it was incorporated into the PDA flask containing concentrated potato dextrose agar media. Thus, after incorporation of 500 ml extract (20%) in 500 ml PDA the final concentration of the powdered extract into the PDA was remained 20%.

Fresh extracts (Crude): The fully expanded leaves of *Aloe vera* were selected from the plants, washed with distilled water and were subjected to surface sterilization with 70% ethyl alcohol followed by 0.1% $HgCl_2$. The parenchymatous covering of the leaves were peeled and the gel drained out. Slurry was formed with the help of sterilized pestle and mortar

Ethanol extracts: For the preparation of ethanol extracts, Twenty grams powder was soaked in 200 ml. of the solvents namely ethanol for 24 hours. The contents were then filtered through Whatman filter paper no. 1 and the filtrate was evaporated to dryness. This dried extract was further powdered and then dissolved in distilled water. Acetone extract was prepared in a similar manner except that the extracted powder was dissolved in 0.15 N NaOH and was further neutralized with 0.15 N HCl. For standardization of the concentration of the effective form the crude and powder extracts were used @ 20% ,30% ,40% and 50% concentrations while the ethanol extract was used @ 1, 2, 3 and 4 percent concentrations, respectively. All the leaf extract forms were subjected to antifungal activity assay with crude (20%), powdered (20%), and ethanol (1%) extracts and was further subjected to poisoned food technique. Among all the tested extracts the effective extract against the respective pathogens were selected and again subjected to different four concentration of crude, powder (20,30,40, and 50 %) and ethanol extract at (1,2,3and4 %) to find out its appropriate form respectively. The effective forms under different concentrations (crude and powder extract at 40% and 50%, ethanol at 3and 4%) were further evaluated against test pathogens. On the basis of significance, the concentrations of respective forms were standardized against the pathogens and were also compared with the recommended chemicals *viz.*, carbendazim (0.1%) and mancozeb (0.2%), under *in-vitro* condition. The plant extracts were amended aseptically to melted potato dextrose agar medium in appropriate proportions and sterilized at 121°C, 15Ib/inch^2 pressure for 15 minutes and allow cooling. Twenty ml of the medium was poured in each 10 cm diameter Petridish and solidified. One disc (7 mm) of the medium containing fungal culture of the pathogen was cut from the 7 days old culture and was transferred in the centre of the Petri dish under aseptic condition. There were five replicates of each treatment. The plates were then incubated at temperature 28±2°C. Average radial growth of the pathogen in mm was recorded after 7 days of incubation and data analyzed statistically to observe the difference among various treatments. Petridishes containing media devoid of the plant extract but with the medium without plant extract but with same amount of distilled water serve as control.

RESULTS AND DISCUSSION

Result (Table 1) shows that among the three forms of *Aloe vera* leaf extracts two forms (crude and ethanol) significantly inhibited the growth of fungal mycelium but none of them could absolutely inhibited the growth, however the crude form was found effective against eight phytopathogenic fungi *viz.*, *Rhizoctonia solani, Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii, Colletotrichum gloeosporioides, Alternaria solani, Alternaria alternata, Tolyposporium penicillariae,* and *Alternaria brassicae.* The ethanol extract also effectively inhibited the growth of *Phoma sorghina* and *R.bataticola*. In respect of growth inhibition the crude extract was significantly superior over the other forms.Powder extract was least effective. Among the different forms, the crude extract (20%) was more effective than powder and ethanol extracts against eight fungal pathogens viz. *Rhizoctonia solani, Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii, Colletotrichum gloeosporioides, Alternaria solani, Tolyposporium penicillariae, Rhizoctonia bataticola* and *Alternaria brassicae*. It was again tested against the above fungal pathogens under four different concentrations viz., 20, 30, 40 and 50% to find out the appropriate concentration.

The result (Table 2 a) revealed that all the four concentrations significantly inhibited the growth

Table 1: Efficacy of different forms of *Aloe vera* leaf extracts against different fungal pathogens (means of 2 years)

Treatments	Radial growth of fungal mycelium (mm)									
	Rizoctonia Solani	Fusarium Oxysporium f.sp.ciceri	Sclerotium rolfsii	Phoma sorghina	Colletorichum gleosprioides	Alternaria solani	Alternaria alternate	Tolyposporiu mpenicillariae	R. bataticola	Alternaria brasicae
Crude @20%	82.0	33.6	35.0	33.5	46.4	36.2	38.2	26.1	46.5	30.3
Powder 20%	85.2	46.4	46.2	51.6	63.8	66.5	45.2	36.6	60.3	53.3
Ethanol@1%	87.0	44.0	43.8	28.4	56.3	56.8	36.4	48.3	48.3	48.2
Control	90.0	78.0	88.3	73.0	82.0	85.0	83.6	76.0	76.0	86.0
CD(P= 0.05)	3.36	4.69	2.44	4.28	5.21	5.13	1.73	7.72	7.72	5.35

of above fungal pathogens but none of the concentration absolutely inhibited the growth, however its affectivity against all the above tested fungal pathogens increases with the increase in the concentration from 20 to 50%. The growth of *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *ciceri* under 50% concentration of crude *Aloe vera* leaf extract was 68.60 mm and 10.60 mm, respectively as compared to 90.00 and 75.00 mm of control while 71.00 and 19.00 mm fungal growth of the above two pathogens was recorded under its 40% concentration.

Table 2a: Comparative efficacy of crude extracts of *Aloe* vera leaf under four concentrations against test Pathogens

Treatments	Radial growth of fungal mycelium (mm)							
	Rizoctonia Solani	Fusarium Oxysporium f.sp.ciceri	Sclerotium rolfsii	Colletorichum gleosprioides	Alternaria solani	Tolyposporiu mpenicillariae	R. bataticola	Alternaria brasicae
Crude extract @ 20%	83.6	59.0	50.6	50.6	56.0	46.4	48.0	
Crude extract @ 30%	76.0	39.2	4.3	27.4	33.0	28.0	28.5	30.3
Crude extract @ 40%	71.0	19.0	19.0	11.0	22.0	16.0	21.0	22.0
Crude extract @ 50%	68.6	10.6	9.2	8.1	11.0	8.3	16.0	15.0
Control	90.0	75.0	0.0	84.0	83.9	90.0	73.5	84.0
CD (P= 0.05)	5.41	4.91	6.07	4.99	6.14	4.45	4.33	6.58

However, 50% showed significant inhibition over 40%, indicating indicate that there is a need to test the higher concentrations (>50%) of *Aloe vera* leaf extract under crude form to obtain complete inhibition of fungal growth against *Rhizoctonia solani* and *Fusarium oxysporum* f.sp. *ciceri*.

Table 2b: Comparative efficacy of Ethanol extracts of *Aloe* vera leaf under four concentrations against two Pathogens

Treatments	Radial growth of fungal mycelium (mm)	
	Phoma sorghina	Alternaria alternata
Ethanol extract@ 1%	53.33	49.5
Ethanol extract@ 2%	27.53	29.0
Ethanol extract@ 3%	21.33	20.2
Ethanol extract@ 4%	15.43	16.0
Control	74.33	83.7
CD (P= 0.05)	7.7	4.4

Similarly 50% concentration of *Aloe vera* leaf extract under crude form showed significant inhibition of *Sclerotium rolfsii, Colletotrichum gloeosporioides, Alternaria solani, Tolyposporium penicillariae, Rhizoctonia bataticola* and *Alternaria brassicae* over its 40% concentration. These also reflect to go for its higher concentration to obtain the complete/better inhibition. Though the minimum growth of *Colletotrichum gloeosporioides* and *Tolyposporium penicillariae* was recorded under 50% concentration, but it was statistically at par with its 40% concentration.

The fungitoxicity of ethanol form (Table 2 b) of *Aloe vera* leaf @ 4% was significantly higher over its 3% concentration for the inhibition of *Phoma sorghina* and *Alternaria alternate.* On the basis of significance, the effective concentrations of respective forms were standardized against the pathogens and were also compared with the recommended chemicals under *in-vitro* condition. The results (Table 3) revealed that the growth of *Rhizoctonia solani, Fusarium oxysporum f.sp. ciceri, Sclerotium rolfsii, Colletotrichum gloeosporioides*, *Alternaria solani, Tolyposporium penicillariae, Rhizoctonia bataticola* and *Alternaria brassicae* was effectively inhibited by the crude extract of *Aloe* vera leaf but the crude extract even at 50% concentration was significantly less effective than carbendazim (0.1%) and mancozeb (0.2%). It means that the crude

extract @ 50% concentration may not act as an alternative to the carbendazim (0.1%) and mancozeb (0.2%), however as per the effectively trend it seems that they may act as an alternative source to the chemical under much higher concentration. Like crude extract, the ethanol extract also effectively inhibited the growth of *Phoma sorghina* and *Alternaria alternata,* but these extracts could not show absolute inhibition under 1 to 4% concentration and were significantly inferior over carbendazim (0.1%) and mancozeb (0.2%), though the effectivity of ethanol extract was gradually increased with the concentration from 1 to 4%. It indicates that the absolute inhibition in the growth of above test fungi may be achieved under >4% concentration of ethanol form of *Aloe* vera leaf extract.

Table 3: Comparison of *In-vitro* efficacy of different forms of *Aloe* vera leaf extracts with chemicals against test pathogens

Treatments	Radial growth of fungal mycelium (mm)									
	Rizoctonia Solani	Fusarium Oxysporium f.sp.ciceri	Sclerotium rolfsii	Phoma sorghina	Colletorichum gleosprioides	Alternaria solani	Alternaria alternate	Tolyposporium penicillariae	R. bataticola	Alternaria brasicae
Crude @ 40%	74.0	27.0	29.4	N.E	28.6	31.0	N.E	33.4	38.6	37.0
Crude @ 50%	69.1	18.4	20.6	N.E	18.5	20.8	N.E	16.0	21.6	23.9
Powder @ 40%	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
Powder @ 50%	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E	N.E
Ethanol extract @ 3%	N.E	N.E	N.E	28.3	N.E	N.E	6.0	N.E	N.E	N.E
Ethanol extract @ 4%	N.E	N.E	N.E	10.3	N.E	N.E	6.4	N.E	N.E	N.E
Mancozeb @ 0.2%	2.0	0.0	6.0	0.0	5.0	0.0	0.0	0.0	7.1	0.0
Carbendazim @ 0.1%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Control	90.0	70.1	67.0	81.0	82.5	83.3	85.0	86.0	85.0	84.0
CD (P= 0.05)	3.39	2.59	2.72	2.82	2.85	3.08	3.33	2.89	3.60	3.98

The above results revealed that the crude extract of *Aloe vera* leaf was more effective than its other forms for the inhibition of *Rhizoctonia solani, Fusarium oxysporum* f.sp. *ciceri, Sclerotium rolfsii, Colletotrichum gloeosporioides, Alternaria solani, Tolyposporium penicillariae, Rhizoctonia bataticola* and *Alternaria brassicae.* Ethanol form was more effective against *Phoma sorghina* and *Alternaria alternata.* The above forms in the concentration of up to 50% were less effective than the carbendazim @ 0.1% and mancozeb @ 0.2%. Therefore, in the direction of eco-friendly management there is a need to test the extracts against the fungal pathogens under much higher concentration so that possibilities may be created for the use of the above forms of *Aloe vera* extract as an alternative to the chemical for the management of above fungal pathogens (Shivpuri and Gupta 2001, Alam *et al.* 2002 and Ahmed *et al.* 2004).

REFERENCES

Ahmed, S. S. and Azhar, S.W. Iqbal (2004) Antifungal activity of Allium, Aloe, and Solanum species. *Pharmaceutical Biology*, 42(7): 491-498.

Ahmed, N. and Grainge, M. (1982) Some promising plants for pest control under small scale forming operation in developing countries working paper resource system institute, *East-West Centre, Honolulu, Hawai, USA*. pp 55.

Alam, Shahidul, Banu, Sabina, M., Ali, M.; Forman, Akhter, Nargis, Islam, M. Rafiqul and Alam, M.S. (2002) *In vitro* Inhibition of conidial germination of *Colletotrichum gloeos-porioides* Penz. by fungicides, plant extracts and phytohoromones. *Pakistan Journal of Biological Science*. 5(3): 303-306.

Anonymous (2006) For *Aloe vera* - A Semi Finished Products like gel powder, like *Aloe vera* Drink or Fizzy Tablet. *Technology Transfer and Project Management Network. Ensymm Consulting for Biotechnology*.

Eggli, U. (2001) Illustrated handbook of succulent plants: Monocotyledons, Springer. *Reynolds Botanical Journal of Linnaean Society*, 19: 157.

Grindlay, D. and Reynolds, T. (1986) The *A. vera* phenomenon: a review of the properties and modern uses of the leaf parenchyma gel. *Journal of Ethnopharm*, 16: 117–151.

Haller Jr., J.S. (1990) A drug for all seasons: medical and pharm. History of *Aloevera Bulletin of New York Academy of Medicine*. 66: 647–659.

Shivpuri, Asha and Gupta, R.B.L. (2001) Evaluation of different fungicides and plant extracts against *Sclerotinia sclerotic-orum* causing stem rot of mustard. *Indian Phytopath*., 54(2): 272-274.