IJBPAS, December, 2015, 4(12): 6850-6858

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

ANTIFUNGAL POTENTIAL OF *CYNODON DACTYLON* AGAINST GREY MOLD DISEASE

FAIZA KARIM, *KHAJISTA JABEEN AND SUMERA IQBAL

Department of Botany, Lahore College for Women University, Jail Road, Lahore, Pakistan

***Corresponding Author: E Mail: khajista_1@hotmail.com**

ABSTRACT

In present study the antifungal potential of *Cynodon dactylon* L. was evaluated against *Botrytis cinerea* Pers. Methanolic extract of *C. dactylon* was prepared and its various concentrations of 0.25%, 0.5%, 1% and 1.5% were tested against *B. cinerea*. All the concentrations of this extract significantly reduced the colony growth of *B. cinerea* by 86% to 72%. Phytochemical analysis of *C. dactylon* revealed the presence of tannins, flavonoids, saponins, glycosides and phlobatannins while alkaloids and terpenoids were absent. Methanolic extract of *C. dactylon* was subjected for fractional guided bioassays. Different organic fractions of *C. dactylon* extract were isolated viz. n-hexane, chloroform, ethyl acetate and n-butanol. Minimum inhibitory concentration (MIC) of these isolated fractions and synthetic fungicide was recorded for each fraction at interval of 24, 48 and 72 hours. The MIC of various concentrations from (100 mg – 0.19 mg mL^{-1}) was recorded for each fraction at interval of 24, 48 and 72 hours. Chloroform, ethyl acetate and synthetic fungicide were found most effectual in retarding conidial germination of test fungus with MIC of 0.19 mg mL^{-1} after 72 hours of incubation period. This study concluded that methanolic extract of *C. dactylon* contains antifungal agents against *B. cinerea.*

Keywords: Antifungal, grey mold, methanolic extract, MIC

INTRODUCTION

Botrytis cinerea Pers. is an airborne plant pathogen with a necrotrophic lifestyle attacking over 200 crop hosts worldwide. *B. cinerea* belongs to class ascomycetes is responsible for grey mould on hundreds of dicot plants (Elad *et al.*, 2004). Gray mold disease affect leaves, stems, flowers, fruits and even tubers and roots (e.g. potatoes and

carrots in storage). At high humidity the typical brownish-grey, furry, fungal growth with huge numbers of spores are produced.

There are many methods to control this destructive fungus such as cultural, chemical, biological etc. One of the frequently used methods is crop rotation with non-susceptible crops such as alfalfa or small grains which suppress the built-up of sclerotia in soil (Mishra *et al*., 2012). Effective management of a plant disease is a key to save plants from diseases caused from microbes, since plants are significant as they are both economical and aesthetic. Fungal plant diseases are usually controlled by application of fungicides. Some flavonoids, diterpenoids, monoterpenoids, stilbenes, steroidal glycoalkaloid, and triterpenoids have some effect against this fungus. Chemicals idinamine has also been traditionally used to control Botrytis (Vio-Michaelis *et al*., 2012). However extensive use of chemicals leads to bio hazardous effects on ecosystem and their persistent applications lead to resistance in pathogens against these fungicides (Prasad *et al*., 2010).

Post-harvesting diseases with naturally occurring pathogens can be controlled biologically. Use of biologically active natural products to control plant diseases in many fields has become an alternative of chemical fungicides (Nunes *et al.,* 2002). Plants constitute a rich source of bioactive chemicals and most important of these bioactive constituents are terpenes, alkaloids flavonoids and phenolic compounds (Arumugam *et al.*, 2014).

Cynodon dactylon (L.) belongs to the family of Poaceae is a valuable herbal medicine and used (Kanimozhi and Ratha, 2012). *C. dactylon* is used as an important ingredient in various ayurvedic preparations such as anabolic, antiseptic, astringent, cyanogenetic, demulcent, depurative, laxative, diuretic and emollient (Abdullah and Chong, 2012).

The objective of this study was to investigate the antifungal potential of *Cynodon dactylon* to control *B. cinerea.*

MATERIAL AND METHODS

Experimental material collection

Cynodon dactylon was collected from the Lahore College for Women University, Lahore. The plant material was dried under sunlight and stored in polythene bags. Test fungus *Botrytis cinerea* was isolated from the diseased onion bulb. This culture was sub cultured and maintained on 2% MEA (malt extract agar) and kept at 4 C in refrigerator.

Antifungal bioassay

Fifty grams of dried plant material was soaked in 200 mL (methanoland left for one week at room temperature. Material was filtered through an autoclaved muslin

cloth after one week. The final volume of organic solvent extract was reduced to 2.2 g by evaporating at room temperature and diluted by adding 11 mL of distilled water to make 20% of stock solution. This stock extract was stored in refrigerator at 4 ºC and used within 4 days.

ME (Malt Extract) 2% medium was made in 250 mL flask by adding 1.2 g of Malt extract in 60 mL of distilled water and autoclaved at 121 ºC and 15 lb $inch^{-2}$ for 30 minutes. Four concentrations of plant material of viz. 0.25%, 0.5%, 1%, 1.5% v/v concentrations of organic solvent extract was made by thoroughly mixing of 0.75, 1.5, 3, and 4.5 mL of stock solutions and 59.25, 58.5, 57, 55.5 mL in the malt extract medium to make total volume up to 60 mL. Control treatments were without any plant extracts. Each concentration was endowed with 50 mg of Chloromycetin capsules to avoid bacterial contamination. Then from each flask further three replicates were prepared.

In vitro antifungal bioassay was conducted with methanolic extract. Mycelial discs (5 mm) was prepared using sterilized cork borer from the tip of seven days old culture of *B. cinerea* and was placed in the center of each flask which are then covered with foil paper to avoid contamination. After 7 days fungal biomass in each flask was filtered and dried to constant weight in an electric oven and weighed. Percentage growth inhibition of the fungal colonies was calculated by applying the following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

Phytochemical analysis

The bio-chemical constituents of *C. dactylon* were identified by the phytochemical analysis of the methanolic plant extract. Standard technique and methodology of (Edeogea *et al.*, 2005; Parekh and Chanda, 2007) were used in this assay.

Bioassay guided fractionation

Fifty gram powdered plant material of *C. dactylon* was thoroughly extracted with 150 mL of methanol (MeOH) at room temperature. The extract was then allowed to evaporate at room temperature for 4 days. After 4 days a gummy mass of 3 g was obtained and this methanolic extract was then portioned between n-hexane and water using separating funnel. The aqueous fraction of methanolic extract was consecutively partitioned with chloroform, ethyl acetate and n-butanol (Jabeen *et al.*, 2013). This partitioning gives four different fractions of n-hexane, chloroform, ethyl acetate and n-butanol in increasing order of polarity. These partitions were then further allowed to evaporate at room temperature for 4 days. After 4 days gummy mass of n-hexane (1 g), chloroform (1 g), ethyl

acetate (0.5 g) and n-butanol (0.1 g) were obtained.

Minimum inhibitory concentration (MIC) of the isolated fractions

The antifungal activity of four organic solvent fractions viz. n-hexane, chloroform, ethyl acetate and n-butanol of methanolic extract of *C. dactylon* was investigated against *B. cinerea* by Minimum Inhibitory Concentration (MIC) bioassays. The MIC values of these fractions along with a reference synthetic fungicide Puslan were tested in test tubes by the serial micro dilution assay (Jabeen *et al*., 2011). The highest concentration was prepared by dissolving 100 mg of each extract into 1 mL distilled water and 1 mL DMSO (Dimethyl sulfoxide), this concentration was further serially diluted and the minimum applied concentration was 0.19 mg mL^{-1}. Freshly prepared ME medium was added to seven days old fungal culture of *B. cinerea* to reach a final conidial concentration $1x10^5$, 100 µL of this was added to test tubes having a diameter of 1.6cm and 15cm long. Test tube containing DMSO and distilled water was used as control. These test tubes were incubated at 25-30 ºC. The MIC of the fractions was observed visually after 24, 48 and 72 hours by using inverted microscope to study the fungal mycelia growth.

Statistical analysis

Data was analyzed statistically by applying ANOVA followed by Duncan's multiple range tests to separate the treatments means at $P \leq 0.05$ significant level by using Co-stat software (Steel *et al*., 1997).

RESULTS AND DISCUSSION

The present study was conducted to evaluate *in vitro* antifungal activity of *C. dactylon* for the control of *B. cinerea*. According to the results *C. dactylon* shows high antifungal activity against *B. cinerea*. All the applied concentrations i.e. 0.25, 0.5, 1 & 1.5% of methanol extracts of whole plant of *C. dactylon* significantly inhibited the growth of test fungus (Fig. 1). However, 86% reduction in *B. cinerea* was observed in 1.5% concentration. Previously Bazie *et al*. (2014) reported that methanolic fraction of *C. dactylon* show high antifungal activity against *Colletotrichum musae* and this activity is due to the presence of bioactive compounds.

The phytochemical analysis of *C. dactylon* showed the presence of tannins, flavonoids, saponins and phlobatannins glycosides while terpenoids and alkaloids were absent in test plant *C. dactylon* (Table. 1). Previously, many researchers phytochemically analyzed the *C. dactylon* and their results coincide with our findings (Asthana et al., 2012; Arumugam *et al*., 2014).

The results from MIC show that all the applied concentration (100– 0.19 mg mL^{-1}) of four organic solvents, n-hexane, chloroform, ethyl acetate and n-butanol show marked variation in their antifungal activity against *B. cinerea* which might be due to the difference in the solubility of different compounds of *C. dactylon* in different solvents (Table. 2). According to our findings chloroform, ethyl acetate and fungicide were found highly effective against *B. cinerea* with MIC of 0.19 mg mL^{-1}. It might show that the compound with antifungal property might be soluble in ethyl acetate and can be further extracted for the production of fungicides. Fraction of n-hexane also show antifungal property and can also be tested. There are many reports in literatures which support our findings. Yanar *et al*. (2011) also proved it to be antifungal against *Phytophthora infestans* and according to the results those compounds might be present in ethyl acetate fraction. Rekha and Shivanna (2014) study the *Aspergillus fumigatus* associated with *C. dactylon* produced antifungal metabolites. Abdullah *et al*. (2014) screened the potential antifungal activity from some phytochemical compounds of *C. dactylon* against *Ganoderma boninense*. Smitha *et al*. (2014) also suggest that, the plant widely available could be a prominent source of medicinally important natural compounds. Rao *et al*. (2011) study the potential antibacterial and antifungal activity of aqueous extract of *C. dactylon*.

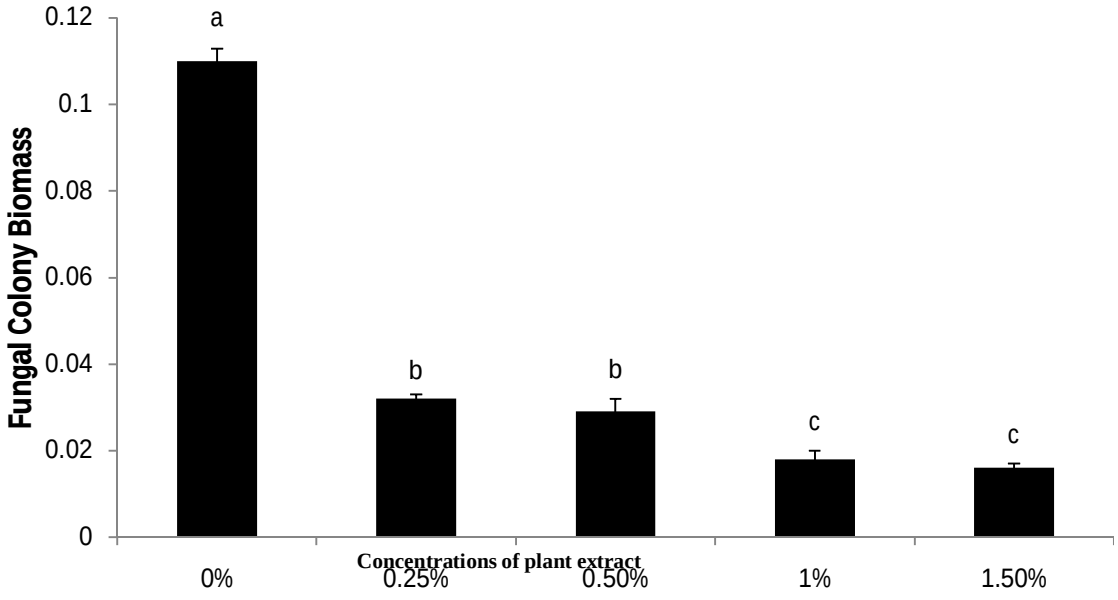

Fig. 1: Effect of Methanolic extract of whole plant of *Cynodon dactylon* on *Botrytis cinerea*. Vertical Bars show standard error of mean of three replicates. Values with different letters show significant difference as determined by DMR test

Table 1: Phytochemical constituents of *Cynodon dactylon*

Sr. No.	Phytochemicals constituents	Observations
1.	Tannins	+
2.	Phlobatannins	+
3.	Saponins	+
4.	Alkaloids	_
5.	Glycosides	+
6.	Terpenoids	-
7.	Flavonoids	+

(+ indicate the presence and – the absence)

Table 2: Table 1: MIC values of different organic fractions isolated from *C. dactylon* and fungicide against *B. cinerea* after 24, 48 and 72 hours incubation periods

Fractions	Concentration (mg mL^{-1})									
	5	2.5	1.25	0.625	0.3125	0.156	0.078	0.039	0.019	
24 hours after incubation										
Control(H_2O)	-	-	-	-	-	+	+	+	+	
Control (DMSO)	-	-	-	-	-	-	-	+	+	
n-Hexane	-	-	-	-	-	-	-	-	-	
Chloroform	-	-	-	-	-	-	-	-	-	
Ethyl acetate	-	-	-	-	-	-	-	-	-	
n-Butanol	-	-	-	-	-	-	-	-	-	
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	
48 hours after incubation										
Control(H_2O)	+	+	+	+	+	+	+	+	+	
Control (DMSO)	+	+	+	+	+	+	+	+	+	
n-Hexane	-	-	-	-	-	-	-	+	+	
Chloroform	-	-	-	-	-	-	-	-	-	
Ethyl acetate	-	-	-	-	-	-	-	-	-	
n-Butanol	-	-	-	-	-	-	-	+	+	
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	
72 hours after incubation										
Control(H_2O)	+	+	+	+	+	+	+	+	+	
Control (DMSO)	+	+	+	+	+	+	+	+	+	
n-Hexane	-	-	-	-	-	-	-	+	+	
Chloroform	-	-	-	-	-	-	-	-	-	
Ethyl acetate	-	-	-	-	-	-	-	-	-	
n-Butanol	-	-	-	-	-	-	+	+	+	
Metalayl+Mancozeb	-	-	-	-	-	-	-	-	-	

CONCLUSION

Present study concluded that *C. dactylon* posses strong antifungal potential against grey mould disease. Further, more extensive biological evaluations and chemical characterization on isolation of the active principles from *C. dactylon* is needed.

REFERENCES

[1] Abdullah S, Gobilik, J. and Chong K. P. 2012. Preliminary phytochemical study and antimicrobial activity from various extract of *Cynodon dactylon* (L.) Pers. (Bermuda) against selected pathogens. *International Journal of*

Pharmacy and *Pharmaceutical Sciences*, 4: 227-30.

[2] Arumugam, N. T., Boobalan, P., Rajeswari, R. and Duraimurugan, M. D. 2014. Antimicrobial activity and Phytochemical Screening of *Cynodon dactylon* and *Carica papaya*. *Research in Biotechnology*, **5**(5): 21-31.

[3] Asthana, A., Kumar, A., Ganwar, S. and Dora, J. 2012. Pharmacological perspectives of *Cynodon dactylon*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **3**(2): 1135-1147.

[4] Bazie, S., Ayalew, A. and Woldetsadik, K. 2014. Antifungal Activity of Some Plant Extracts against (*Colletotrichum Musae*) the Cause of Postharvest Banana Anthracnose. *Journal of Plant Pathology and Microbiology*, **5**(2): 2-4.

[5] Elad, Y., Williamson, B., Tudzynski, P. and Delen, N. 2004. *Botrytis spp*. and diseases they cause in agricultural systems – an introduction. Botrytis: *Biology, Pathology and Control*, Kluwer Academic Publishers. Dordrecht. Netherlands. 1-6.

[6] Edeogea, H. O., Okwu, D. E. and Mbaebie, B. O. 2005. Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, **4**(7): 685-688.

[7] Jabeen, K., Javaid A., Ahmad E., and Athar M. 2011.Antifungal Compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei*, the cause of chickpea blight. Nat. Prod. Res. 25: 264-76.

[8] Jabeen, K., Hanif, S., Naz, S. and Iqbal, S. 2013. Antifungal activity of *Azadirachta indica* against Alternaria solani. *Journal of Life Science and Technology*, **1**(1): 89-92.

[9] Kanimozhi, D. and Ratha bai, V. 2012. Evaluation of Anti-Microbial Activity of *Cynodon dactylon. International Journal of Research in Pharmacy and Science*, **2**(2): 34-43.

[10] Mishra, R. K., Adholeya, A. and Sardana, H. R. 2012. *Integrated Pest Management*: *Strategies for Onion and Garlic*. *The Energy and Resources Institute*, New Dehli.

[11] Nunes, C., Usall, J., Teixido, N., Torres, R. and Vinas, I. 2002. Control of *Penicillium expansum*

and *Botrytis cinerea* on apples and pears with the combination of *Candida sake* and *Pantoea agglomerans*. *Journal of Food Protection*, **65**: 178-184.

[12] Prakash, B. and Solanki, R. 2011. *Cynodon dactylon* (L.) Pers.: A Valuable Medicinal Plant. *Research Journal of Medicinal Plant*, **5**(5): 508-514.

[13] Parekh, J. and Chanda, S. V. 2007. *In vitro* Antimicrobial activity and Phytochemical analysis of some Indian Medicinal Plants. *Turkish Journal of Biotechnology*, **31**: 53-58.

[14] Prasad, M. N., Bhat, S. and Sreenivasa, M. Y. 2010. Antifungal activity of essential oils against *Phomopsis azadirachtae*-the causative agent of die-back disease of neem. *Journal of Agricultural Technology*, **6**(1): 127-133.

[15] Rekha, D. and Shivanna, M. B. 2014. Diversity, antimicrobial and antioxidant activities of fungal endophytes in *Cynodon dactylon* (L.) Pers. and *Dactyloctenium aegyptium* (L.) P. Beauv. *International journal of current microbiology and applied sciences*, ISSN: 2319-7706, **3**(8): 573-591.

[16] Rao , A. S., Nayanatara A. K., Kaup, R. S., Sharma, A., Kumar, A. B., Vaghasiya, B. D., Kishan, K. and Pai, S. R. 2011. Potential Antibacterial and Antifungal activity of aqueous extract of *Cynodon dactylon*. *International journal of pharmaceutical sciences and research*, **2**(11): 2889-2893.

[17] Smitha, K. R., Ruveena, T. N., Arif, R. and Mesna, A. 2014. Exploring antimicrobial and antioxidant activity of a traditional herbal medicine, *Cynodon dactylon* L. *Pers. and its phytochemical screening*. *World Journal of Pharmacy and Pharmaceutical Sciences*, **3**(3): 1744-1757.

[18] Steel, R. G. D., Torrie, J. H. and Dickey, D. A. 1997. Principles and Procedures of Statistics: A Biometrical Approach, New York, USA: McGraw Hill Book Co., Inc (ISBN 0070610282).

[19] Vio-Michaelis, S., Hidalgo, G. A. Gomez, M., Vera, R. P. and Montenegro, G. 2012. Antifungal activity of three Chilean plant extracts on *Botrytis cinerea*.

Botanical Sciences, **90** (2): 179-183.

[20] Yanar, Y., Kadioglu, I., Gokce, A., Demirtas, D., Goren, N., Cam, H. and Whalon, M. *In vitro* antifungal activities of 26 plant extracts on mycelial growth of *Phytophthora infestans* (Mont.) de B Bary. *African Journal of Biotechnology,* **10**(14): 2625-2629.