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Antifungal Activity of Some Plant Extracts on *Alternaria burnsii*: The Causal Agent of Alternaria Blight of Cumin

Actividad antifúngica de algunos extractos de plantas en *Alternaria burnsii*: el agente causal del tizón del comino por *Alternaria*

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ABSTRACT

Cumin is a vital seed spice crop in India. The most dreaded disease in the cumin crop is Alternaria blight, caused by *Alternaria burnsii*. It is a significant production constraint for cultivating the cumin crop in Gujarat as in India. Eight different plant species were utilized for the eco-friendly management of this disease. The experiment on managing Alternaria blight of cumin was conducted at Atmiya University, Rajkot. Crude plant extracts were prepared in water, acetone, and cow urine as solvents at different concentrations (5%, 10%, and 15%). The poisoned food technique determined the in vitro antifungal activity of these plant extracts. Based on in vitro studies, all the plants exhibited significant antifungal activity. During the research work, it was found that the highest inhibition was recorded for Azadirachta *indica* (78.15%) extract prepared in acetone, followed by *Mimuspos elengi* (67.75%) extract prepared in cow urine at 15% concentration (at p≤0.01). The lowest inhibition was recorded for *Aloe barbadensis miller* (40.24%), and *Annona reticulata* (43.48%) extract prepared in water at 15% concentration.

Keywords: Alternaria blight, *Alternaria burnsii*, plant extract, antifungal activity, PDA media, poison food technique.

RESUMEN

El comino es un cultivo vital de semillas de especias en la India. La enfermedad más temida en el cultivo del comino es el tizón de Alternaria, causado por *Alternaria burnsii*. Es una limitación de producción significativa para el cultivo de comino en Gujarat como en la India. Se utilizaron ocho especies de plantas diferentes para el

manejo ecológico de esta enfermedad. El experimento sobre el manejo del tizón del comino por *Alternaria* se llevó a cabo en la Universidad de Atmiya, Rajkot. Se prepararon extractos crudos de plantas en agua, acetona y orina de vaca como solventes a diferentes concentraciones (5%, 10% y 15%). La técnica del alimento envenenado determinó la actividad antifúngica in vitro de estos extractos de plantas. Con base en estudios in vitro, todas las plantas exhibieron una actividad antifúngica significativa. Durante el trabajo de investigación se encontró que la mayor inhibición la registró *Azadirachta indica* (78,15%) extracto preparado en acetona, seguido de *Mimuspos elengi* (67,75%) extracto preparado en orina de vaca al 15% de concentración (a p≤0,01). La inhibición más baja se registró para el extracto de *Aloe barbadensis miller* (40,24 %) y *Annona reticulata* (43,48 %) preparado en agua al 15 % de concentración.

Palabras clave: tizón de *Alternaria* , *Alternaria burnsii*, extracto vegetal, actividad antifúngica, medio PDA, técnica de veneno alimentario.

INTRODUCTION

India is among the most prominent seed spice producers, exporters, and consumers. Among them, cumin, known as Jeera (*Cumin cyminum* L.), is a vital seed spice crop in several subtropical countries and has medicinal value. It belongs to the family Apiaceae and the order Umbellalas (Joshi, N.C. 1955). It is a Rabi crop grown from October- November and harvested from February-March. The climate should be moderately cool and dry. Cumin is mainly cultivated in India, Egypt, Libya, Iran, Pakistan, and Mexico. Cumin is mainly cultivated in Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh, and Bihar (Sharma YK et al., 2013). Sustainable cumin cultivation is continuously challenged by diseases that cause losses in yield. Alternaria blight caused by Alternaria burnsii is the most devastating disease in major cumin-growing areas in Rajasthan and Gujarat (Lodha S.K. et al., 2007). Alternaria burnsii causing blight of cumin was recorded for the first time in Pakistan (Shakir et al.,1995). In India, the blight of cumin caused by A. burnsii was first reported by Uppal et al. (1938). From Rajasthan, it was first reported by Joshi (1955). Alternaria blight is considered a significant constraint in sustainable cumin production, so various control strategies include fungicides, biological agents, botanicals, and their combinations. However, several factors, including pathogenic variability, influence the efficacy of these management practices. Investigations were made to devise an effective management strategy for Alternaria blight of cumin.

MATERIALS AND METHODS

Experimental site: The experiment was conducted at the research lab of Atmiya University, Rajkot in Gujarat, from January to March 2022 to evaluate the antifungal activity of Ardusi, Neem, Aleovera, Custard Apple, Diesel plant, Karanj, Vinca rosea, and bullet wood.

Fungal strains: Pure culture of Alternaria burnsii was acquired from the Plant pathology department of Agriculture University, Junagadh.

Identification of the fungus by slide preparation: Examination of the fungal colony characteristics was done through microscopic examination. A loop of fungus was taken and placed on a sterile glass slide using a sterile needle, and it was stained using lactophenol cotton blue and covered with the coverslip. Then, the microscope was used to examine the morphological characteristics of fungal structures (Grahovac M et al.,2017).

Morphological Characters of *Alternaria burnsii*: The conidia of *Alternaria burnsii* are single or in chains, and they are smooth with a rounded base and pointed towards the apex. Beak is septate or non-septate. *Alternaria burnsii* possesses 3-6 transverse and 1-3 longitudinal septa called *Muriform conidia*. The conidiophore is branched, erect, straight, or somewhat bent (Singh NK, et al,2016).

Sample Collection: Leaves of eight plants were used in the present study to evaluate their antifungal activity (Table 1). Native plants were selected on the bases of antimicrobial properties and abundant availability. The nominated plants are well adapted to the climatic conditions and popular among local natives for their medicinal properties (Gamble, J. S., 1921). Leaves of selected plants were collected from a different area of Rajkot city.

Preparation of aqueous extract: The fresh Leaves of each plant were washed theory with tap water, followed by sterilized distilled water. 100gm of these Leaves were crushed in 100 ml of distilled water in a grinder. The extracts were strained through a muslin cloth to remove plant debris. The filtrate thus obtained was designated as stock solution. 5%, 10%, and 15% plant extract were prepared from the stock solution of each plant Singh, R. K., & Dwivedi, R. S. (1987). Similarly, each plant extract was prepared in water, acetone (95%), and cow urine (untreated).

Antifungal activity assay of botanical extracts by using poison food technique: 1 ml of each plant extract from different concentrations of 5%, 10%, and 15% were added to 15ml sterilized molten potato dextrose agar mediums in sterile Petri plates. A 5 mm diameter of mycelium disc of the 7-day-old grown *Alternaria burnsii* was placed in the center of the Petri plates containing each plant extract. Plates without plant extract served as the negative control. Plates were incubated at 27 ±2°C. Triplicates were maintained for each treatment in this assay. Radial growth of mycelium was measured after 9 days of incubation (Shrivastava et al., 2011). The results were compared with the negative control.

Calculation: The experiment was repeated thrice, and the mean of three readings was taken for calculations. Percent inhibition was determined with the help of mean colony diameter and calculated using the following formula suggested by Vincent (1947).

$$L = [(C – T)/C] \times 100$$

Where L is percent inhibition, C is the control plate's colony radius, and T is the radial growth of *Alternaria burnsii* in the presence of plant extracts (Shivapratap, et al., 1996).

Statistical analysis: One-way analysis of variance (ANOVA) examined the significant differences between results obtained in each experiment at values of $p \leq 0.001$, followed by Tukey's post hoc test at $p \leq 0.05$.

Table 1: List of Plant and part used for evaluation

Sr. No.	Common name	Scientific name	Family	Plant part used
1	Ardusi	*Adhatoda vasica*	*Acanthaceae*	Leaves
2	Neem	*Azadirachta indica*	*Meliaceae*	Leaves
3	Aloe vera	*Aloe barbadensis miller*	*Asphodelaceae*	Leaves
4	Custard Apple	*Annona reticulata*	*Annonaceae*	Leaves
5	Diesel plant	*Jatropha curcas*	*Euphorbiaceae*	Leaves
6	Karanj	*Millettia pinnata*	*Fabaceae*	Leaves
7	Vinca rosea	*Catharanthus roseus*	*Apocynaceae*	Leaves
8.	Borsali (bulletwood)	*Mimuspos elengi*	*Sapotaceae.*	Leaves

RESULTS AND DISCUSSION

The extracts of eight different plants were tested for antifungal activity at concentrations of 5%,10% and 15%. The radial growth of *Alternaria burnsii* was measured and % Inhibition is calculated for each plant (Table.2).

From the experiment it was found that among all the plant extracts bullet wood aqueous extract at all concentrations (5,10, and 15%) gave the highest inhibition of mycelial growth (60.59, 64.67 and 66.71%) and followed by Karanj (45.80,51.35 and 57.71%) respectively. The lowest inhibition of mycelial growth was given by *Aloe vera* at 5,10 and 15% concentration (34.96, 39.14 and 40.24 %) and followed by Custard apple (35.86, 39.97 and 43.48%) respectively (Table 3).

In case of plant extracts which were prepared in acetone, the highest inhibition of mycelial growth was recorded by Neem *at* 5,10 and 15% concentration (68.68, 73.85,78.15%) and followed by Karanj (66.63, 68.01 and 75.07%) respectively. The lest efficacy was recorded by *Aloe vera* at 5,10 and 15% concentration (42.98, 45.81 and 50.73%) and followed by Custard apple (45.03,47.61 and 58.07) respectively (Table 4).

Plant extracts which were prepared in cow urine, the highest inhibition of mycelial growth was recorded by Neem *at* 5,10 and 15% concentration (61.32, 63.71 and 75.73%) and followed by bullet wood (53.53,62.79 and

67.45%) respectively and least efficacy was recorded by *Aloe vera* (39.14, 42.4 and 46.59 %) and followed by Custard apple (40.34, 47.31 and 56.81%) respectively.

Similar effect of other various plant extracts are effective against *Alternaria burnsii* have been reported by several researcher. The aqueous neem leaf extracts inhibited the mycelial growth of *Alternaria burnsii*. (Hassanein et al,2008). Vijayan (1989) reported that the bulb extract of Allium sativum, leaf extract of Neem and flower extract of *Catharanthus roseus* inhibited the spore germination and mycelial growth of *A. burnsii*. Neem formulations Azadirachtin was found effective under in vitro conditions (Shekhawat, et al.,2013). Gangopadhyay et al. (2010) observed the effects of five plant extracts (botanicals) viz., *Aloe vera*, *Calotropis procera*, *Eucalyptus golobulus*, *Azardiratcha indica* leaves and *A. indica* seed kernel on growth and spore germination of A. burnsii . Polra and Jadeja (2011) evaluated 6 phytoextracts against *Alternaria burnsii* . Jadeja and Pipliya (2008) reported that 5% and 10% extract of garlic cloves and ginger rhizomes were most effective resulting in 78.5% and 73% mean inhibition of *A. burnsii* .

Table:2 Evaluation of plant extract in cow urine against *Alternaria burnsii* on PDA media

Plant extract in Cow urine	Concentration Mean of three replicates on PDA media			
	Control	5%	10%	15%
1.*Adhatoda vasica* (Ardusi)				
2.*Azadirachta indica* (Neem)				
3.*Aloe barbadensis miller* (Aleovera)				
4.*Annona reticulata* (Custard Apple)				
5.*Jatropha curcas* (Diesel plant)				
6. *Millettia pinnata* (Karanj)				
7. Catharanthus (Vinca rosea)				
8. Mimuspos elengi (Bullet wood)				

Table-3: In vitro evaluation of antifungal activity of plant extracts in water against *Alternaria burnsii*

Plant extracts	Colony Diameter (mm) *at conc.			Average Colony Diameter (mm)	% Inhibition* ± Standard Deviation			Average % Inhibition
	5%	10%	15%		5%	10%	15%	
Adhatoda vasica (Ardusi)	58.53 ± 0.23	54.76 ± 0.67	50.86 ± 0.29	54.71 ± 0.39	36.12 ± 0.56	41.60 ± 0.21	53.11 ± 0.38	43.61 ± 0.38
Azadirachta indica (Neem)	52.56 ± 0.36	49.93 ± 0.39	46.24 ± 0.89	49.57 ± 0.54	41.59 ± 0.77	44.51 ± 0.34	48.61 ± 073	44.90 ± 0.61
Aloe barbadensis miller (Aloe vera)	57.72 ±0 .45	52.56 ± 0.84	53.78 ± 1.93	54.68 ± 0.74	34.96 ± 0.64	39.14 ± 0.54	40.24 ± 0.48	38.11 ± 0.55
Annona reticulata (Custard apple)	57.48 ± 0.86	54.02 ± 0.64	42.19 ± 0.73	51.23 ± 0.74	35.86 ± 0.93	39.97 ± 0.48	43.48 ± 0.53	39.77 ± 0.64
Jatropha curcas (Diesel plant)	57.26 ± 0.49	50.48 ± 0.32	43.11 ± 0.49	50.28 ± 0.43	36.36 ± 0.57	43.90 ± 0.53	52.08±0.48	44.11 ± 0.36
Millettia pinnata (Karanj)	48.77±0.85	43.78±0.71	38.06±.39	43.53±0.65	45.80±0.72	51.35±0.84	57.71±0.63	51.62 ± 0.73
Catharanthus (Vinca Rosea)	54.03 ± 0.98	48.77 ± 0.60	39.48 ± 0.21	47.42 ± 0.59	39.96 ± 0.83	45.80 ± 0.59	56.12 ± 0.49	47.29 ± 0.63
Mimuspos elengi (Bullet wood)	35.46 ± 0.73	31.78 ± 0.83	29.95 ± 0.38	32.39 ± 0.64	60.59 ± 0.39	64.67 ± 0.61	66.71 ± 0.38	63.99 ± 0.46
Control	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*Mean of three replications, Significant at p≤0.001 level according to Tukey's Post Hoc test

Table-4: In vitro evaluation of antifungal activity of plant extracts in acetone against *Alternaria burnsii*

Plant extracts	Colony Diameter (mm) *at conc.			Average Colony Diameter (mm)	% Inhibition* ±Standard Deviation			Average % Inhibition
	5%	10%	15%		5%	10%	15%	
Adhatoda vasica (Ardusi)	30.03 ± 0.67	28.78 ± 0.76	25.65 ± 0.32	28.15 ± 0.58	63.26 ± 0.78	67.19 ± 0.84	71.80 ± 0.61	67.41 ± 0.74
Azadirachta indica Neem)	44.84 ± 0.45	23.53 ± 0.34	19.65 ± 0.87	29.34 ± 0.55	68.68 ± 0.38	73.85 ± 0.49	78.15 ± 0.27	73.56 ± 0.38
Aloe barbadensis miller (Aloe vera)	51.30 ± 0.67	48.2 ± 0.65	44.33 ± 0.48	47.94 ± 0.60	42.98 ± 0.59	45.81 ± 0.34	50.73 ± 0.37	46.50 ± 0.43
Annona reticulata (Custard apple)	49.46 ± 0.24	47.15 ± 0.29	34.26 ± 0.54	43.62 ± 0.35	45.03 ± 1.08	47.61 ± 0.53	58.07 ± 0.74	50.23 ± 0.45

Jatropha curcas (Diesel plant)	46.50 ± 0.89	38.64 ± 0.52	32.23 ± 0.29	39.12 ± 0.56	48.33 ± 0.43	57.05 ± 0.36	64.18 ± 0.86	56.52 ± 0.55
Millettia pinnata (Karanj)	33.06 ± 0.46	29.51 ± 0.19	22.43 ± 0.53	28.33 ± 0.39	66.63 ± 0.79	68.01 ± 0.86	75.07 ± 0.39	69.90 ± 0.68
Catharanthus (Vinca Rosea)	44.11 ± 0.54	41.10 ± 0.21	31.94 ± 0.74	39.05 ± 0.49	50.97 ± 0.29	53.32 ± 0.46	64.5 ± 0.73	56.26 ± 0.49
Mimuspos elengi (Bullet wood)	35.46 ± 0.28	31.78 ± 0.57	29.95 ± 0.69	32.39 ± 0.51	60.59 ± 0.53	64.67 ± 0.21	66.71 ± 0.29	63.99 ± 0.34
Control	90.00 ± 0.0	90.00 ± 0.0	90.00 ± 0.00	90.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*Mean of three replications, Significant at p≤0.001 level according to Tukey's Post Hoc test

Table-5: In vitro evaluation of antifungal activity of plant extracts in cow urine against *Alternaria burnsii*

Plant extracts	Colony Diameter (mm) *at conc.			Average Colony Diameter (mm)	% Inhibition* ±Standard Deviation			Average % Inhibition
	5%	10%	15%		5%	10%	15%	
Adhatoda vasica (Ardusi)	51.29 ± 0.79	34.61 ± 0.61	30.98 ± 0.35	38.96 ± 0.58	43.0 ± 0.47	61.53 ± 0.27	65.57 ± 0.18	56.7 ± 0.30
Azadirachta indica Neem)	47.77 ± 0.67	32.65 ± 0.21	21.84 ± 0.88	34.08 ± 0.58	61.32 ± 0.87	63.71 ± 0.49	75.73 ± 0.21	66.92 ± 0.52
Aloe barbadensis miller (Aloe vera)	53.68 ± 0.54	51.84 ± 0.87	48.06 ± 0.73	51.19 ± 0.71	39.14 ± 0.28	42.40 ± 0.73	46.59 ± 0.17	42.71 ± 0.39
Annona reticulata (Custard apple)	54.76 ± 0.64	47.41 ± 0.12	38.86 ± 0.83	47.01 ± 0.53	40.34 ± 0.16	47.31 ± 0.38	56.81 ± 0.38	48.15 ± 0.30
Jatropha curcas (Diesel plant)	48.77 ± 0.29	40.13 ± 0.92	35.86 ± 0.16	41.58 ± 0.45	45.77 ± 0.28	55.40 ± 0.28	60.18 ± 0.32	53.78 ± 0.29
Millettia pinnata (Karanj)	44.35 ± 0.98	34.75 ± 0.38	31.16 ± 0.73	36.75 ± 0.69	46.91 ± 0.17	61.38 ± 0.37	65.37 ± 0.83	57.88 ± 0.45
Catharanthus (Vinca Rosea)	34.80 ± 0.34	44.92 ± 0.64	35.63 ± 0.68	38.45 ± 0.55	50.71 ± 0.73	50.08 ± 0.28	60.40 ± 0.18	53.73 ± 0.39
Mimuspos elengi (Bullet wood)	41.81 ± 0.85	33.48 ± 0.34	29.28 ± 0.23	34.85 ± 0.47	53.53 ± 0.78	62.79 ± 0.72	67.45 ± 0.71	61.25 ± 0.73
Control	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	90.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

*Mean of three replications, Significant at p≤0.001 level according to Tukey's Post Hoc test

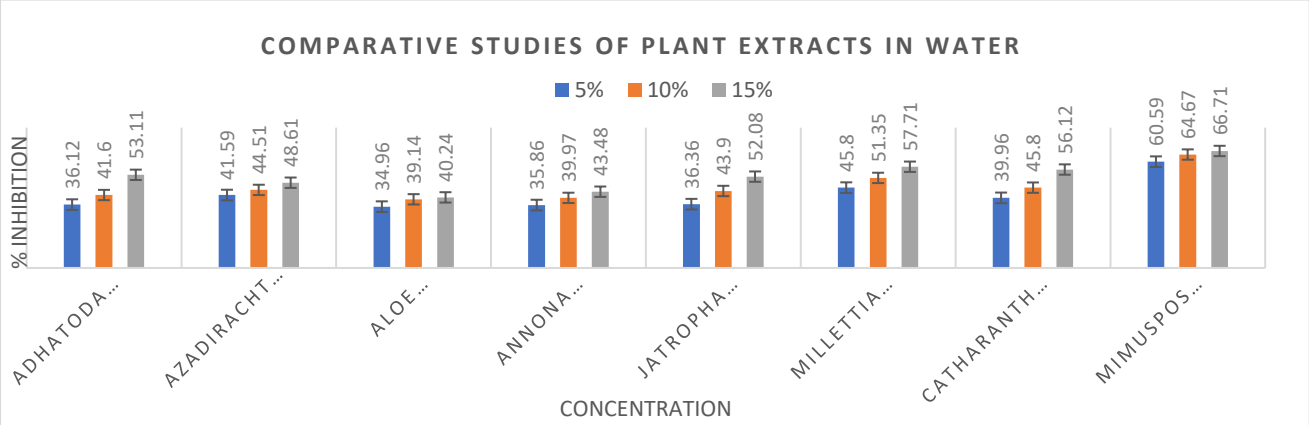

Figure:2 In vitro evaluation of plant extracts in water at different concentration on PDA media.

Significant at p≤0.001 level according to Tukey's Post Hoc test

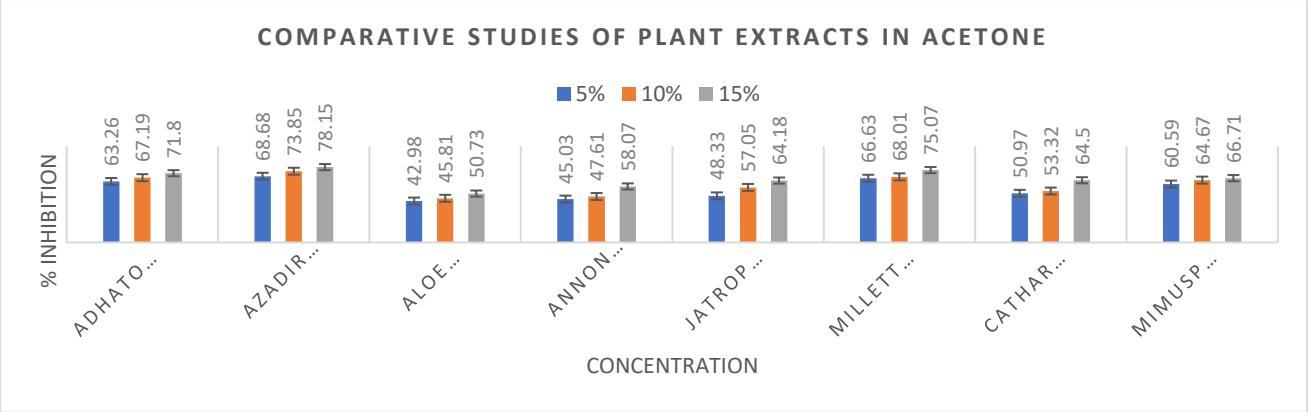

Figure:3 In vitro evaluation of plant extracts in acetone at different concentration on PDA media.

Significant at p≤0.001 level according to Tukey's Post Hoc test

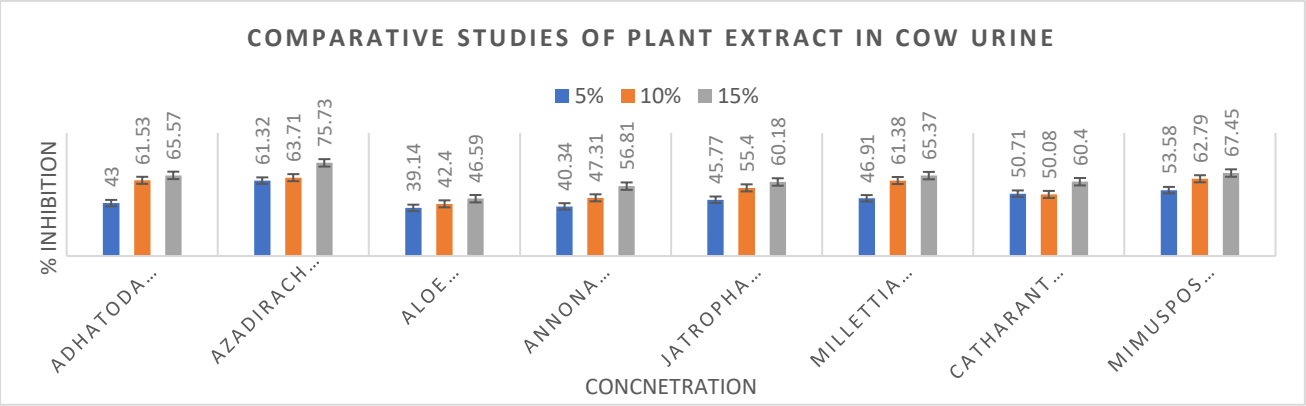

Figure:4 In vitro evaluation of plant extracts in cow urine at different concentration on PDA media. Significant at p≤0.001 level according to Tukey's Post Hoc test

As conclusion, the results of the current study displayed that all selected plant extracts exhibited antifungal activity against the mycelial growth of *Alternaria burnsii*. Extracts prepared in water and cow urine exhibited suitable antifungal activities and could reduce the growth of *Alternaria burnsii*, which is responsible for cumin blight. These preliminary results from in vitro experiments may be supplemented by other more comprehensive studies in the open field (in vivo). This research has concluded that certain plant extracts are a good source of cost-effective and non-hazardous approaches against *Fusarium oxysporum f.sp. cumini*. Plant extracts such as *Azadirachta indica*, *Millettia pinnata*, and *Mimuspos elengi* have good antifungal efficacy, which may be used for formulating new, safer, and eco-friendly natural fungicides. This work has allowed selecting the best plant which may be used as a phyto-fungicide to control crop diseases, with the ultimate goal of developing a green alternative to synthetic fungicides.

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