Effect of Leaf Extract of *Cassia fistula* on the Growth and Development of *Colletotrichum gloeosporioides* and *Cercospora bataticola*

ABSTRACT

Because of the widespread and uncontrolled use of traditional fungicides, many plant pathogens have become resistant to them, making it more challenging to control most fungal plant diseases with these fungicides. Since the dawn of time, phytochemicals have contributed significantly to human welfare without causing any harm. Therefore, PFT method with different leaf extracts of *Cassia fistula* (Aqueous, methanol and acetone) and their three concentrations (250µl, 500µl and 1000µl per 15 ml of PDA) was used against *Colletotrichum gloeosporioides* and *Cercospora bataticola*, causing fruit rot and leaf spot of beet respectively. Results of this study showed that maximum inhibition (76.8%) of *Colletotrichum gloeosporioides* was found with1000µl concentration of methanolic extract of *Cassia fistula* leaves 3 DAI while maximum inhibition of *Cercospora bataticola* (68.7%) was found with the same concentration of acetone extract of *Cassia fistula* leaves 3 DAI. Considerable growth inhibition (68.7%) of *Colletotrichum gloeosporioides* and 50.5% of *Cercospora bataticola* were found with 1000µl concentration of methanolic and acetonic extracts of *Cassia fistula* leaves respectively 5 DAI. Acetonic extract of *Cassia fistula* leaves with 1000µl concentration was also found as a good antifungal for *Colletotrichum gloeosporioides* which inhibited 64.8% and 54.6% 3 and 5 DAI respectively. However, there was no considerable growth inhibition of both fungal plant pathogens with aqueous extract. The results of present study revealed that methanolic and acetonic leaf extracts of *Cassia fistula* can be used for fungal plant disease management with further extensive studies.

Keywords: Aqueous extract, Methanolic extract, Acetone extract, *Colletotrichum gloeosporioides*, *Cercospora bataticola,*
Cassia fistula

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Most of plant diseases are caused by fungi for which farmers use large amount of fungicides to reduce the losses caused by them. Many private pesticide companies (Indian and foreign) are manufacturing thousands of tons of fungicides annually for control of plant diseases. A big part of these fungicides is imported from foreign companies. These fungicides not only control our plant diseases but also pollute the environment and creating a number of problems to the human beings. Due to continuous and indiscriminate use of these fungicides, many plant pathogens have got resistance against these fungicides and most of fungal plant diseases have become difficult to control through conventional fungicides. Plants are "nature's chemical factories", providing the nature's richest source of chemicals on the Earth (Dhaliwal and Koul, 2011). Phytochemicals have been in nature for millions of years without any adverse effects to the ecosystem. These phytochemicals have been documented in ancient Greek, Roman and Indian writings. For thousands of years, people in India placed neem leaves in their beds, books, grain bins, cupboards and closets. Many plant-pathogenic fungi; *Botrytis cinerea* (Wilson et.al. 1970), *Ascochyta rabiei* causing chickpea blight (Jabeen et.al. 2012), *Sclerotium rolfsii*, causing damping off in green house conditions (Derbalah et.al.2012), *Alternaria solani* (Hada and Sharma

2014), *Alternaria alternata, Colletotrichum gloeosporioides and Fusarium moniliforme* (Bhuyan et.al. 2015),
Aspergillus niger, Aspergillus parasiticus, Colletotricum gloeosporioides, Penicillium janthinellum, P. expansum,
Trichoderma harzianum and Fusarium oxysporum (Mahlo et.al. 2016), *Fusarium* (Salhi et.al. 2017), were
tested against different plant extracts. Out of many plants used by ancient Indians *Cassia fistula* (amaltas) is a
medium sized deciduous or semi-deciduous, tropical and subtropical legume tree used as an ornamental,
fodder, fuel, timber and medicine. In Indian literature, this plant has been described to be useful against skin
and liver diseases and in the treatment of haematemesis, pruritus, leukoderma and diabetes,
(Balashanmugam *et.al*. 2014). In Ayurveda system of medicine every part of this plant is recognized for its
medicinal properties. *Cassia fistula* contains alkaloids, terpenoids, reducing sugars, saponins, tannins,
carbonyl, phlobatanin, and steroids (Kulkarni *et.al.* 2015). Leaf extract of *Cassia alata* was found most effective
against *Trichophyton rubrum* and *Microsporum gypseum.* whereas the leaf extract of *Cassia fistula* was found
most potent inhibitor of *Penicillium* (Phongpaichit *et.al.,2004*). Tannin found in *Cassia fistula* is toxic to fungi,
bacteria & yeasts (Scalbert 1991). Alkaloids and flavonoids are considered most potential phytochemicals for
fungal growth inhibition. All these compounds have found to have therapeutic and antimicrobial properties and
have been proven in past researches (Bhalodia *et.al*. 2011, Bhalodia and Shukla 2011, Kushawaha and
Agrawal 2012, War *et.al.* 2014, Prabagar *et.al.* 2020). In many studies, phytochemicals in the leaves of *Cassia*
fistula were found highly active against *Aspergillus terreus* (Kadhim *et.al.* 2016) and for *Rhizopus stolonifer,*
Pencillium digitatum, Pencillium notatum and *Aspergillus niger* (Sharma and Rai. 2019). CFTI-1 and CFTI-2
trypsin inhibitors purified from seeds of *Cassia fistula* were also inhibited growth parameters and
developmental stages of *Helicoverpa armigera* (Pandey *et.al*. 2016). Since Madhya Pradesh is rich in forest
cover area and *Cassia fistula* is abundantly found in these forests therefore, present study, “Effect of leaf
extract of *Cassia fistula* on the growth and development of *Colletotrichum gloeosporioides* and *Cercospora*
bataticola” was carried out to manage plant diseases caused by these fungi and was also aimed to reduce the
use of chemical fungicides to protect our ecosystem from residual effect and developing resistance in plant
pathogens through continuous use these fungicides.

2. Material and Methods / Experimental Details / Methodology (Arial, Bold, 11 font, left aligned, caps)

2.1: Isolation, identification and maintenance of fungal Plant Pathogens

Anthracnose of citrus and leaf spot of beet are major problems of fruit sellers and farmers. *Colletotrichum gloeosporioides* was isolated from citrus fruit infected with anthracnose in the month of September 2020 as – spot of infected lemon fruit was wiped with a cotton swab dipped in 70% ethanol then small pieces (5-10mm size) were digged out with the help of flamed scalpel and forceps. These small pieces were placed on PDA (potato dextrose agar) and incubated for 3-5 days at 27_{0} C for fungal growth.

Cercospora bataticola was isolated from beet (*Beta vulgaris*) crops grown in commercial farm of AKS University, Satna in January 2021. For isolation of *C. bataticola*, small pieces (5-10 mm size) were cut from infected leaf of beet and surface sterilized in 0.1% HgCl solution for 1-2 min then these infected leaf pieces were washed 3-4 times through distilled water. Surface sterilized leaf pieces were placed on solid surface of PDA in petri plates (3 pieces/petri plate) and incubated for 3-5 days at 27_{0} C for fungus development. These two fungal pathogens were purified through subculturing method after identification and were maintained as pure culture in slants containing PDA. The identification of these two fungal pathogens up to species level were done macro and microscopically according to their specific characters.

2.2: Leaf Extraction of *Cassia fistula*

Leaves of *C. fistula* were collected in the month of November, 2020 from Govt. Horticulture nursery, Saleha, Satna. Leaves were washed, dried and were powdered by mechanical grinder. Aqueous Extract was obtained as the method described by Patel (2014) in which 100 g. of leaf powder was homogenized in 250 ml. distilled water (1:2.5 w/v) for 24 hrs. then it was filtered through double layer muslin cloth and again filtrate was filtered through Whatman no. 1 filter paper.

Two organic solvents i.e., Methanol and Acetone. were used in Soxhlet extraction for extraction of phytochemicals from *C. fistula* leaf powder. A thimble was prepared by 0.5 mm Whatman filter paper. A 100 gm leaves powder was equally packed into two thimbles. Then one thimble was loaded into the Soxhlet apparatus and 250 ml. each solvent (Methanol and Acetone) was poured into the Soxhlet. The cassia leaf powder was extracted with solvent for the period of about 48 hours in which 72 cycles were completed and the leaf powder become colourless. Two extracts (Methanol and Acetone) were taken into separate beakers and organic solvents (Methanol and Acetone) were evaporated in water bath to get a syrupy consistency.

2.3: Evaluation of Antifungal Properties of *Cassia fistula* Leaf Extracts

PFT (Poison Food Technique) was used to test the efficacy and dilutions of leaf extracts of *C. fistula*. Different dilutions (250µl, 500µl & 1000µl/15 ml PDA) of leaf extracts (aqueous, methanol and acetone) were prepared in PDA. These different dilutions of three extracts of *Cassia fistula* leaves were poured in a set of three petri plates (as replicates) for each dilution and were allowed for solidification. Fungal samples (*Colletotrichum gloeosporioides* and *Cercospora bataticola*) were inoculated on separate petri plates after solidify poisoned food (Mixture of PDA and leaf extracts of *C. fistula*). Control petri plates (without leaf extract) were also inoculated on the same time for comparison of antifungal activity of the extracts. After inoculation all petri plates were sealed with parafilm to avoid contamination and incubated in BOD incubator at 27°C.

2.4: Measurement of growth Inhibition

Bidirectional (horizontal and vertical) diameters separately of all incubated petri plates were measured (in mm) 3 days after inoculation (DAI) and 5 DAI with the help of a millimeter scale and an average diameter of all replicates was calculated by as following –

Average diameter of a fungal colony = $\frac{Horizontal\ diameter\ +\ vertical\ diameter}{2}$

Again, average diameter of three replicates = $\frac{Average\ diameters\ of\ three\ replicates\ (R1+R2+R3)}{3}$

The antifungal activity was calculated in the term of percentage by using formula below-

Percent of Inhibition = $\frac{(C-T)}{C} \times 100$

Where, C = Diameter of fungal mycelial growth (mm) in control

T = Diameter of fungal mycelial growth (mm) in treated with leaf extract of *C. fistula*

3. RESULTS AND DISCUSSION

3.1: Isolation, identification and maintenance of fungal Plant Pathogens

Colletotrichum gloeosporioides, causing fruit rot of citrus (lemon) was identified as it produced tan to darkened brownish (light tan colour), more or less circular, flat area (spots) on the upper surface of citrus fruit, (Fig. 1). The spot become enlarged and covered the most surface of the lemon fruit. White mycelial and conidial growth was also present in the center of spot which might be conformed as anthracnose disease of citrus caused by *C. gloeosporioides.* When PDA in the petri plates was inoculated with the fungus from the centre of the spot and incubated for 3-5 days, it produced round or nearly round, white to off-white, with dense whitish mycelia distributed as stripes with concentric ringed colonies. The reverse colour of the fungal colonies was creamy yellow to dull yellow. Melanin pigmentation was absent in the colonies (Fig. 2A & B).

Fig. 1. Anthracnose of Lemon; infected fruit with anthracnose disease(A) and conidia of *Colletotrichum gloeosporioides*(B).

Fig. 2. *Colletotrichum gloeosporioides* colony grown on PDA; front view (A) and reverse view (B).

When temporary slides from isolated fungus were observed under compound microscope with magnification of 45X it was seen that conidiophores were cylindrical, long hyaline with rounded tips on which apically single conidia was born at a time. These conidia were elongated, hyaline with round ends which characteristically were slightly narrower in the middle than at the ends, smooth, and aseptate. Teleomorph (asci and ascospores) stage was absent. Therefore, isolated fungus from infected lemon fruit was confirmed as *C. gloeosporioides* (Fig. 1B).

Cercospora bataticola fungus was first identified in the beet crop as it produced circular leaf spots that had a reddish margin. The center of the lesions was start off a light brown and turned to grey after the fungus begins to sporulate. These typical symptoms might be conformed as leaf spot disease of beet caused by *C. bataticola.* For isolation of the fungus, infected tissues of beet leaf were cut into small pieces of 0.5×10 mm and surface sterilized in 0.1% mercuric chloride solution for 2-3 min. then these leaf tissues were washed 3-4 times with distilled water and were placed on PDA surface in petri plates which were allowed to incubation for 3-5 days at 27^0C. After incubation it produced olivaceous, smooth, erumpent and regular, even margin colonies and sparse to moderate aerial mycelium (Fig. 4A & B). The fungus was confirmed up to species level under compound microscope with magnification of 45X and found that mycelium was well-developed, branched, septate, slender, and brown coloured. Conidiophores were septate, dark-coloured on which Conidia were developed on geniculate structures. These conidia were hyaline or pale yellow, solitary, acicular, straight to curved, 25-150 × 2-4.5 µm, hyaline, 2-12-septate with acute apex.

Fig. 3. *Cercospora bataticola* colony grown on PDA; front view (A) and reverse view (B).

After identification up to species level both fungal plant pathogens (*C. gloeosporioides* and *C. bataticola*) were maintained in slants containing PDA as pure culture for further use.

3.2: Evaluation of Antifungal Properties of *Cassia fistula* Leaf Extracts

Zone inhibition of *C. gloeosporioides* and *C. bataticola* fungal colonies by different leaf extracts of *Cassia fistula* was taken in consideration for antifungal activities. The results of the antifungal activities of *Cassia fistula* are presented in Table 1 and 2.

Table 1. Antifungal activity of leaf extract of *Cassia fistula* against *Colletotrichum gloeosporioides.*

Coc. of
ext./15m

l PDA	DAI	Aqueous extract				Methanol extract				Acetone extract			
		Colony dia. (mm)		Mean dia. (mm)	% Inh.	Colony dia. (mm)		Mean dia. (mm)	% Inh.	Colony dia. (mm)		Mean dia. (mm)	% Inh.
		Hori	Vert.			Hori.	Vert.			Hori	Vert.		
250µl (T_1)	3DAI	32.0	30.0	31.0	07.5	31.0	25.0	28.0	16.4	38.0	34.0	36.0	18.2
	5DAI	53.0	53.0	53.0	00.9	46.0	50.0	48.0	10.3	54.5	54.5	54.5	09.2
500µl (T_2)	3DAI	34.0	32.0	33.0	01.5	20.0	16.5	18.2	45.5	26.5	27.0	26.7	39.2
	5DAI	52.0	52.0	52.0	02.8	33.0	30.5	31.7	40.6	47.5	43.5	45.5	24.2
1000µl (T_3)	3DAI	33.0	34.0	33.5	0	09.0	06.5	07.7	76.9	15.5	15.5	15.5	64.8
	5DAI	51.0	52.0	51.5	03.7	20.0	13.5	16.7	68.7	27.5	27.0	27.2	54.6
Control (C)	3DAI	35.0	32.0	33.5	0	35.0	32.0	33.5	0	44.0	44.0	44.0	0
	5DAI	53.0	54.0	53.5	0	53.0	54.0	53.5	0	60.0	60.0	60.0	0

According to the results in Table 1, it was found that three concentrations of aqueous extract of *Cassia fistula* leaves i.e., 250µl, 500µl and 1000µl inhibited the growth of *C. gloeosporioides* 7.5, 1.5 and 0 percent respectively. Same concentrations of methanol extract inhibited the growth of *C. gloeosporioides* 16.4, 45.5 and 76.9 percent respectively; while acetone extract inhibited the growth of this fungus by 18.2, 39.2 and 64.8 percent respectively as compared to control after three days after inoculation (DAI) and the growth of *C. gloeosporioides* was inhibited by these concentrations of aqueous, methanol and acetone extracts of *C. fistula* leaves by 0.9, 2.8 and 3.7 percent; 10.3, 40.6 and 68.7 percent and 9.2, 24.2 and 54.6 percent respectively 5 DAI

(Table 1 and Fig. 4 & 5). The result of antifungal activity (Fig. 6) shown that maximum growth inhibition of *C. gloeosporioides* was found with methanol extract while there was no significant difference in growth inhibition of *C. gloeosporioides* with aqueous extract.

Fig. 4. Effect on growth and development of *Colletotrichum gloeosporioides* 3 DAI By leaf extracts of *Cassia fistula*; fungus growth without any extract (C, control), fungus growth in aqueous extract (A.), fungus growth in methanolic extract (B.) and fungus growth in acetone extract (C.).

Fig. 5. Effect on growth and development of *Colletotrichum gloeosporioides* 5 DAI By leaf extracts of *Cassia fistula*; fungus growth without any extract (C, control), fungus growth in aqueous extract (A.), fungus growth in methanolic extract (B.) and fungus growth in acetone extract (C.).

Fig. 6. Effect of DAI and leaf extracts of *Cassia fistula* on growth and development of *Colletotrichum gloeosporioides*.

Table 2. Antifungal activity of leaf extract of *Cassia fistula* against *Cercospora bataticola.*

Coc. of ext./15 ml PDA	DAI	Aqueous extract				Methanol extract				Acetone extract			
		Colony dia. (mm)		Mean dia. (mm)	% Inh.	Colony dia. (mm)		Mean dia. (mm)	% Inh.	Colony dia. (mm)		Mean dia. (mm)	% Inh.
		Hori.	Vert			Hori.	Vert			Hori.	Vert.		
250µl (T_1)	3DAI	23.5	23.0	23.2	03.1	22.0	20.5	21.2	11.4	15.0	18.0	16.5	31.2
	5DAI	43.5	42.0	42.7	05.0	43.5	42.5	43.0	04.4	33.0	30.5	31.7	29.4
500µl (T_2)	3DAI	21.0	19.0	20.0	16.7	18.5	18.5	18.5	22.9	14.5	13.5	14.0	41.7
	5DAI	42.5	41.0	41.7	07.2	36.5	35.0	35.7	20.5	30.0	28.0	29.0	35.5
1000µl (T_3)	3DAI	16.5	16.0	16.2	32.3	12.5	12.5	12.5	47.9	07.5	07.5	07.5	68.7

5DAI 34.5 37.0 35.7 20.5 32.5 32.5 32.5 27.8 22.0 22.5 22.2 50.5
Control
(C)
3DAI 24.0 24.0 24.0 0 24.0 24.0 24.0 0 24.0 24.0 24 0
5DAI 45.0 45.0 45.0 0 45.0 45.0 45.0 0 45.0 45.0 45 0

The results of growth inhibition of *C. bataticola* (Table 2) with three concentrations of aqueous, methanol and acetone extract of *C. fistula* leaves i.e., 250µl, 500µl and 1000µl were found as 3.1, 16.7 and 32.3 percent; 11.4, 22.9 and 47.9 percent and 31.2, 41.7 and 68.7 percent respectively 3 DAI as compared to control and 5.0, 7.2 and 20.5 percent; 4.4, 20.5 and 27.8 percent and 29.4, 35.5 and 50.5 percent respectively 5 DAI (Fig. 7 & 8).

The maximum growth inhibition of *C. bataticola* was found with Acetone extract while minimum growth inhibition of *C. bataticola* was found with Aqueous extract (Fig. 9).

Fig. 7. Effect on growth and development of *Cercospora bataticola* 3 DAI By leaf extracts of *Cassia fistula*; fungus growth without any extract (C, control), fungus growth in aqueous extract (A.), fungus growth in methanolic extract (B.) and fungus growth in acetone extract (C.).

Fig. 8. Effect on growth and development of *Cercospora bataticola* 5 DAI By leaf extracts of *Cassia fistula*; fungus growth without any extract (C, control), fungus growth in aqueous extract (A.), fungus growth in methanolic extract (B.) and fungus growth in acetone extract (C.).

Fig. 9. Effect of DAI and leaf extracts of *Cassia fistula* on growth and development of *Cercospora bataticola*.

By looking present condition of the crop diseases and their control methods, integrated disease management has become necessary to face the problems related to plant diseases and their management. When we go through “Vraksha Ayurveda” written by Sur Pal we know that almost all plant diseases can be controlled by either plant products or animal bi-products.

Antifungal activities of *Cassia fistula* leaf oil against *Rhizopus stolonifer, Penicillium digitatum, P. notatum* and *Aspergillus niger* were evaluated by Sharma and Seema in 2019. Crude methanolic leaf extract of *C. fistula* inhibited 50% growth of *Penicillium marneffei* (Phongpaichit *et.al.,2004*). Essential oil of *Cinnamomum impressinervium* exhibited 80% antifungal activity against *Colletotrichum gloeosporioides* (Bhuyan et.al. 2015). Aqueous extract of *Cassia fistula* leaves and bark inhibited 64.3% growth of *Aspergillus* fungus (Prabagar *et.al.* 2020). In present study methanol extract of *Cassia fistula* leaves, inhibited maximum

growth of *C. gloeosporioides* (76.8%) with 1000µl concentration 3 DAI (Table 1) while maximum inhibition (68.7%) of *C. bataticola* was also found with 1000µl concentration of acetone extract of *Cassia fistula* leaves. Increasing inhibition of both fungi was also found with increasing concentrations of leaf extracts (Fig. 6 and 9). Present study also showed that aqueous extract of *C. fistula* leaves did not inhibit the growth of both fungi (*C. gloeosporioides* and *C. bataticola*). Maximum activity of methanol and aqueous crude extracts of *Cassia fistula* leaves was observed in *A. fumigatus* (Panda *et.al.*2010). The results of present study (Table 1 and 2) showed that there might not be sufficient amounts of phytochemicals extracted in aqueous extract of *C. fistula* leaves which could inhibit fungal growth. Therefore, in present study efficacy of different concentrations and DAI are taken into consideration against plant pathogens. Use of plant products for the treatment of plant pathogenic fungi has some benefits such as bio degradability, availability, low toxicity and cost effectiveness. In present study, organic acid (methanol and acetone) extracts of *C. fistula* leaves showed strong antifungal activity against *C. gloeosporioides* and *C. bataticola.*
As described in result section all three concentrations 250µl, 500µl and 1000µl of methanolic extract showed maximum inhibition against *C. gloeosporioides* and acetonic extract against *C. bataticola.* (Fig. 6 & 9) as tested by PFT method. The aqueous extract showed varied degree of inhibition in comparison to methanol and acetone extracts.

4. Conclusion

It concluded that these extracts are also considered for further research to assess their potential. No one might use/studied the leaf extract of *C. fistula* for management/growth inhibition of plant pathogenic fungi. Therefore, the results of present study cannot be compared with the work done by others. As use of synthetic fungicides available commercially pose immediate threat to environment as well as not deemed fit for consumption therefore, it is the need to look for alternative source to control plant diseases. In this regard, *C. fistula* leaf extract can be considered for further evaluation and alternative as natural fungicide. In future the purified leaf extract of *C. fistula* can be used in crop protection, sustainable and organic farming to reduce cost of production and environmental pollution.

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Competing interests

The authors declare no Competing Interests.

Authors' Contributions S.K.Y. conducted the research & drafted the manuscript, G.S. edited the manuscript, D.S. guided the research work and S.K. compiled data and prepared tables. All authors have read and approved the final manuscript.

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