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Biological control of phytopathogenic fungus *Colletotrichum falcatum* using *Bos taurus* (cow) urine and dung

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Abstract

Present study edge on comparative efficacy and *in vitro* activity of *Bos taurus* (cow) urine and dung for controlling red rot disease of sugarcane by determining percentage mycellial growth inhibition (MGI) of *Colletotrichum falcatum.* The study was carried out on nine isolates of *Colletotrichum falcatum* obtained from, Department of Biotechnology, Uka Tarsadia University. Results showed the percentage inhibition of mycelial growth suppressed and varied greatly with respect to different isolates and days of incubation and kind of bio matters. In case of cow urine, highest percentage (48.76%) MGI was recorded for CHA8 isolate and minimum (26.08%) MGI was recorded for GAN6 isolate. . Efficacy of cow dung was found to be varied greatly for all *C. falcatum* isolates. Cow dung showed 47.59% MGI for cfCHA8 while minimum percent (21.14%) MGI was recorded for cfNAV. The percentage mycelial growth inhibition found to be varied with time of incubation. In most cases the maximum inhibition was recorded after five days of incubation. Present research accomplished that both the bio-matters were found effective in controlling the growth of *C. falcatum* to certain extent. The use of cow urine and dung can be the cost-effective and eco-friendly approach for controlling sugarcane red rot disease.

Keywords: *Red rot disease, Bos taurus dung and urine, Antifungal, Sugarcane, Colletotrichum falcatum.*

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Introduction

Sugarcane (*Saccharum officinarum* L.) is the most important cash crop grown over worldwide in tropical and subtropical regions suffers from many fungal diseases. India is known to be the second largest producer of sugarcane after Brazil in international trade. The Red rot is chiefly a disease of standing sugarcane and is caused by *Glomerella tucumanensis* (Speg.) von Arx and MulIer (conidial stage *Colletotrichum falcatum* Went) (Agnihotri 1996).

This disease retards the yield drastically and deteriorates the juice quantity and quality thus affecting both the farmers and millers (Abbott 1938). The survival of pathogen and the severity of disease in the field depend upon type of sugarcane variety, soil type, environmental condition, etc. Although efforts were made at various angles to tackle this important problem which target the breeding of disease resistance varieties. However the numbers of newly released cultivars surrender to the pathogen almost as soon as they become popular due to the frequent emergence of new variants of the pathogen owing mutation or recombination (Malathi et al. 2013)

Generally farmers used chemical fungicides to control fatalities in the field due to fungal diseases like wilt and red rot in sugarcane. It is estimated that these diseases would become more reasonably significant as fungicides are hazarders to human health as well as herbivorous animals. In addition to that many fungal pathogens could acclimatize to surmount and become resistant towards these fungicides.

Panchagavya is a term used to describe five major substances, obtained from cow, which include cow's urine, milk, ghee, curd and dung, possesses medicinal properties against many disorders and are used for the medicinal purpose singly or in combination with some other herbs (Joseph and Sankarganesh 2011). Among the panchagavya, cow urine and dung has got several applications in agriculture. It is revealed to have an inhibitory activity against many phytopathogenic fungi and bacteria such as *C. capsici*, *Rhizoctonia solani*, *Phytophthora nicotianae*, *Sclerotinia sclerotiorum*, *Fusarium solani*, *S. rolfsii*, Staphylococcos aureus, *Staphylococcos epidermitis*, *Bacillus subtilis*, *Klebsiella pneumoniae* and *Proteus vulgaris*

(Jarald et al. 2008; Sapna 2013). The use of cow urine has been demonstrated in Ayurveda such as Charakasamhita and Shushrutasamhita. Cow urine extracts of plants and cow urine in combination with phytoextract were reported to have antimicrobial activity (Akhter et al. 2006; Rakesh et al. 2013). Antifungal activity of cow urine distillate was also reported against clinical pathogens like *Aspergillus niger* and *Aspergillus flavus* (Sathasivam et al. 2010). In addition to antimicrobial activity, cow excreta also add the organic matter to the soil and support the growth of plants. The potential of utilizing panchagavya as biofertilizer was tested on the pulses *Vigna radiata, Vigna mungo, Arachis hypogea, Cyamopsis tetragonoloba, Lablab purpureus, Cicer arietinum* and the cereal *Oryza sativa* by growing in soil amended with dried traditional and seaweed based panchagavya (Sangeetha and Thevanathan 2010). In present study we have determined the antifungal activity of cow urine and dung against the sugarcane red rot pathogen, *Colletotrichum falcatum*.

Materials and Methods

Test pathogens

In this study *Colletotrichum falcatum* isolates viz., cfNAV, cfVES, cfPAR, cfTIM, cfMAR, cfGAN, cfKAM, cfCHA and cfMAD were obtained from Department of Biotechnology, Uka Tarsadia University. All the isolates were maintained on oat meal agar slant at 4 ^{0}C in refrigerator.

Collection of cow urine and cow dung

Cow urine and dung was collected in a sterile container from a local cow variety at early morning. The urine was taken to the lab followed by filteration through Whatman No. 1 and stored in airtight container for further work.

Antifungal Activity of Cow Urine and Cow Dung against C. falcatum

In present study antifungal activity of cow urine and cow dung against *C. falcatum* isolates were determined on cow urine oatmeal agar (CUOMA) and cow dung oat meal agar (CDOMA) by poison food technique. The CUOMA medium was prepared from 750ml oat flakes extract and 250ml of cow urine. The CDOMA was prepared by addition of 2% cow dung in oat meal agar. Then media were sterilized in autoclave at 120°C for 15 min. Each Petri dish was poured with 20 ml sterilized medium for solidification and used for the further experimental processes. Mycelial discs of 5 mm in diameter were transferred from the margins of the 7 day old grown colony of each test isolate to the centre of CUOMA, CDOMA and OMA plates. OMA plates were served as control. Inoculated plates were placed in plastic bags and incubated at 28±2°C in the dark. There were three replicate plates of each medium per isolate. In incubator plates were arranged in a random complete block design. The diameter of the each of the test isolates was recorded at 3, 5 and 7 d after inoculation in centimeters at two axes perpendicular to one another. Average colony diameter in centimeters was recorded and percent Inhibition of mycelial growth was calculated using formula given below (Patel et al. 2015).

$$\% \text{ MGI} = (\text{MGC-MGT})/\text{MGC} \times 100 \qquad (1).$$

Where, MGI = Mycelial growth inhibition, MGC = Mycelial growth in control subtracting the diameter of inoculum disc, MGT = Mycelial growth in treatment the subtracting diameter of inoculum disc.

Results and Discussion

The present study was carried out to determine inhibitory effect of cow dung and cow urine against various isolates of *Colletotrichum falcatum*. The growth of all nine isolates on different media at different time intervals in days after inoculation is presented in Figure1. The growth of *C. falcatum* isolates was recorded in terms of mm unit. It was revealed from the data the mycelial growth of all test fungus were found to be reduced on oat meal agar plate poisoned with cow urine and dung. Experiments on cow urine alone or combination of cow urine with plants showed inhibition of *Sclerotinia sclerotiorum*, well known to cause *Sclerotinia* rot in cucumber (Basak et al. 2002b), *Fusarium solani* f.sp. *Cucurbitae* causing root rot disease of cucumber (Basak et al. 2002a), *Bipolaris sorokiniana* causing leaf blight of wheat (Akhter et al. 2006), *Xanthomonas oryzae* pv. *oryzae* causing leaf blight of paddy (Murugan et al. 2012) and *Fusarium oxysporum* f.sp. *zingiberi*, *Ralstonia solanacearum* and *Pythium aphanidermatum* causing rhizome rot of ginger (Rakesh et al. 2013). Jabin (2003) reported that cow urine showed antifungal activity against *F. semitectum* and cow urine mixed with leaf extracts of *C. procera, V. negundo* and *C. alata* completely (100%) inhibited the mycelial growth of the pathogen.

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In present study, results showed that cow urine significantly decreased the mycelial growth even after 7 days of incubation in comparison to cow dung. Mycelial growth inhibition recorded and it was found to be varied after 3, 5 and 7 day of incubation (Table 1). In case of cow urine, in most of the treatments maximum activity was recorded after 5 days of incubation, after that decrement in the cow urine activity was observed. It may be due to the evaporation of some volatile components from cow urine. Cow urine has bioactive properties that enable it to be a fairly potent antibacterial, antioxidant, antihelminthic, anticancer, and antifungal agent (Gotora et al. 2014). Cow urine at different concentrations reported to had significant effect on overall growth characteristics of *F. lateritium* (Gotora et al. 2014).

Overall comparison indicates that the cow urine had more potential to reduce the mycelial growth as compare to cow dung. Among the entire test organism, the minimum growth of *C falcatum* isolates were recorded in media supplemented with cow urine. The growth of cfVES isolate was found to be retarded by 46.24% on CUOMA media after three days of incubation compare to the control while the same fungus showed the prominent growth on the seventh day after incubation and the recorded mycelial growth inhibition was found to be 20.25%. In case of percentage mycelial growth inhibition by cow urine, the highest percentage inhibition (56.52%) was observed in cfCHA isolates after 3 days of incubation followed by 5 days (52.59%) and 7 days (37.18%) period of incubation (Figure 1).

On the flipside cow dung had shown highest mycelial growth suppression (54.93%) of cfCHA isolates after three days inoculation. Both the media CUOMA and CDOMA showed maximum mean mycellial growth inhibition of cfCHA isolate i.e., 48.76% and 47.59%, respectively which indicates cfCHA strain would be more susceptible. However the percentage mycelial growth inhibition of cfNAV isolate was recorded as 28.87% and 21.14% both on CUOMA and CDOMA, respectively, compare to all other isolates. Morphologically both of these fungus were differ from each other as cfNAV

Table 1: Effect of cow urine and dung supplemented media on the mycellial growth inhibition (%) of different isolates of *C. falcatum* **(P< 0.05).**

C. falcatum isolates	Mycelial growth inhibition (%)							
	Cow Urine				Cow Dung			
	3rd day	5th day	7th day	Mean	3rd day	5th day	7th day	Mean
cfNAV	23.72	36.58	26.33	28.87	8.27	7.41	16.81	21.14
cfVES	46.24	38.69	20.25	35.06	5.68	11.54	18.01	25.06
cfPAR	22.47	37.87	25.7	28.68	9.18	16.77	19.58	22.89
cfTIM	29.28	40.21	32.37	33.95	42.26	32.15	23.88	33.44
cfMAR	21.51	46.75	39.91	36.05	31.79	30.35	30.03	33.77
cfGAN	10.41	40.35	27.48	26.08	21.81	25.77	23.9	25.11
cfKAM	53.61	42.39	25.69	40.56	51.89	40.98	35.57	41.52
cfCHA	56.52	52.59	37.18	48.76	54.93	45.18	37.99	47.59
cfMAD	34.74	40.29	33.06	36.03	34.07	30.25	40.31	35.53
Mean	33.16	41.74	29.77		28.87	26.71	27.34	

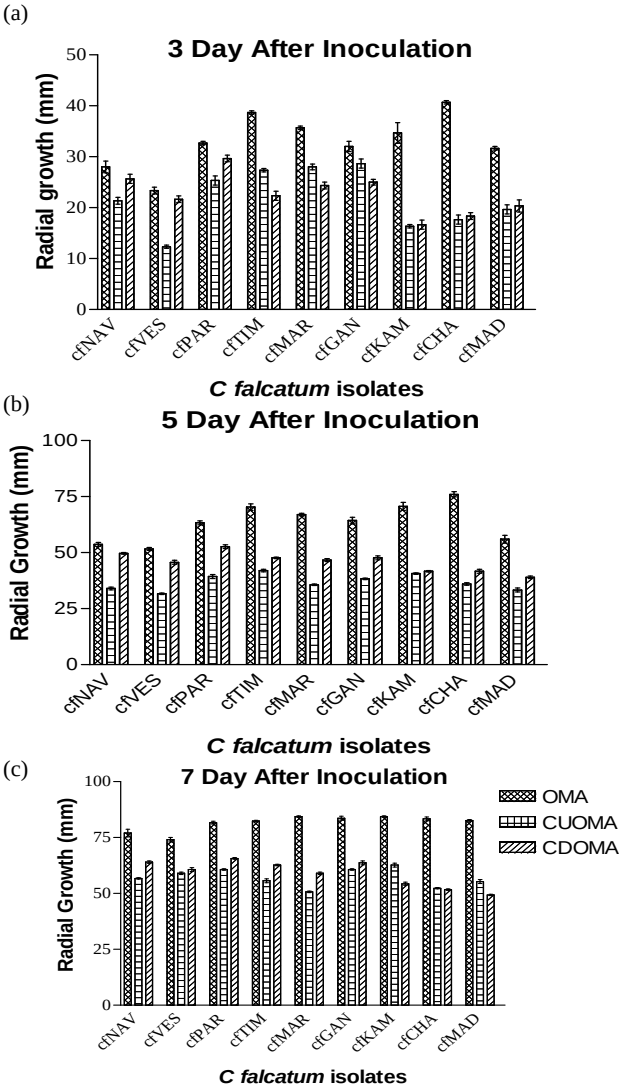

Figure 1: Growth of *C falcatum* isolates on OMA (Oat Meal Agar Plate), CUOMA (Cow Urine Oat Meal Agar) and CDOMA (Cow Dung Oat Meal Agar) at different interval (a) 3 days (b) 5 days and (c) 7 days after inoculation. Bar indicates Standard Error of Mean (n=3).

mature with whitish dense mycelia whereas cf CHA grown with grayish sparse mycelia. These might be the relations between growth pattern and resistance of the fungus.

The results of the current *in vitro* study reveal the potential of cow urine and dung usage as a control measure against red rot disease in sugarcane. It has been reported that cow excreta both cow urine and cow dung are important manure and are natural pesticides. These two natural products can be used as manure by the growers all over the world for the cultivation of wide variety of crops. In addition they have an advantage of being easily available. Modern agricultural experts recommend the use of cow excreta can improve the soil fertility in place of chemical fertilizer which would damage the soil in the long run. *In vivo* trials in field need to be conduct to ascertain cow urine and dung efficiency in the control of *C. falcatum*.

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