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In-vitro response of cow urine as potential Fungitoxic against soil borne plant pathogens of chickpea (*Cicer arietinum* L.)

Priyanka Yadav, Kamal Khilari, Ramesh Singh, Gopal Singh, DV Singh and Akash Tomar

Abstract
Chickpea (*Cicer arietinum* L.), also known as Bengal Gram is one of the major pulse crops cultivated and consumed in India. It is a cheap source of protein (about 17-20%) compared to animal protein. Due to increasing environmental concern and health hazard the old management practices of the diseases in the form of fungicides, need to be replaced with safe and eco-friendly approach. The present investigation was carried out to know the response of cow urine on soil borne plant pathogens of chickpea under *in vitro* conditions. Effect of cow urine at different concentration *viz*. 5%, 10% and 15% was tested against *Fusarium oxysporum* f. sp. *ciceris, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* causing vascular wilt, collar rot and stem rot disease in chickpea respectively. Observations were recorded on inhibition of mycelial growth of the pathogens at different concentrations of cow urine. Under the study 100% inhibition of mycelial growth of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* was recorded in all the tested concentration as compared to control, which was fully covered the petri plate after 144 hours of inoculation. However, in case of *Fusarium oxysporum* f.s p. *ciceris* maximum 50.00 per cent inhibition of mycelium growth were recorded at 15% concentration as compared to control after 192 hours of inoculation.

Keywords: Chickpea, cow urine, concentrations, eco-friendly

Introduction
Chickpea, *Cicer arietinum* (L.) is an important *Rabi* season pulse crop grown and consumed all over the world, especially in the Afro-Asian countries. It is also one of the major pulse crops cultivated and consumed in India and also known as Bengal gram. In India, chickpea accounts for about 45% of total pulses production. Similar to the case of other pulses, India is the major chickpea producing country and contributing for over 75% of total world chickpea production (Maurya and Kumar, 2018) [8]. India is the largest producer of gram production followed by Australia, Myanmar and Ethiopia (FAO STAT, 2019). In India, Bengal gram takes first position in total pulse production followed by black gram (Third Advance Estimates, 2020-21, DES-AP). Uttar Pradesh is the 4th largest producer with an area of 0.5 million hectares with production of 0.57 million tonnes (Anonymous, 2019) [2].
More than 172 pathogens infecting chickpea have been reported worldwide (Nene *et al.*, 1996) [11]. In India, this crop suffers from serious diseases like Fusarium wilt (*Fusarium oxysporum* f. sp. *ciceri*), Dry root rot (*Macrophomina phaseolina* Tassi.) wet root rot (*Rhizoctonia solani* Kuhn.), Black root rot (*Fusarium solani* Mart.), Collar rot (*Sclerotium rolfsii*) Sclerotinia stem rot (*Sclerotinia sclerotiarum* Lib.), bacterial blight (*Xanthomonas campestris* pv. *cassia*), Ascochyta blight (*Ascohyta rabiei*), Botrytis grey mold (*Botrytis cinerea*) and stunt virus (Nene *et al*., 1987) [10]. Among them, Fusarium wilt, collar rot and Sclerotinia stem rot are serious threat to production and productivity of chickpea. These pathogens are mainly soil borne and producing resting spore for survive in soil up to six years even in the absence of the host (Haware *et al.*, 1996) [15].
Use of chemicals continues to be major strategy to mitigate the menace of crop disease. However, because of the environmental concerns and other hazards associated with use of chemicals, use of bio product is gaining importance. In this regard, research on less costly and readily available bio-product pose a limited threat to the environment for management of diseases.

One such cow urine mostly concentrated in the Indian subcontinent where cow products and by products are held in high esteem, have shown that cow urine has bioactive properties that enable it to be a fairly potent antibacterial, antioxidant (Jarald *et al.,* 2008 [6]; Yadav *et al.,* 2008) [14], antihelminthic, anticancer (Jain *et al* 2010) [5], and have antifungal activity (Patil *et al.,* 2007; Kekuda *et al.,* 2010) [12, 7]. Cow urine contains phenolic acids (Gallic, caffeic, ferulic, o-Coumaric, Cinnamic, and salicylic acids) which have antifungal characteristics (Singh *et al.,* 2012) [13]. However, it remains to be established whether the antifungal activity is a result of one or a combination of these phenolic acids. The purpose of this study was to determine the *in vitro* efficacy of cow urine in inhibiting mycelial growth of *Fusarium oxysporum* f. sp. *ciceris, Sclerotium rolfsii* and *Sclerotinia sclerotiorum*, therefore, ascertain its potential use as a low-cost control measure.

Method and Material

Isolation of pathogens

Fusarium oxysporum f. sp. *ciceris, Sclerotium rolfsii* and *Scelerotinia sclerotiorum* were isolated from infected plant part of chickpea that showed symptoms of wilt, collar rot and stem rot respectively. Fresh diseased parts of plants showing disease symptoms were washed thoroughly small pieces of 20-50 mm size were cut from samples with the help of sterilized scalpel in such a way that each of them contained healthy and diseased tissues. These pieces were surface sterilized with1 per cent sodium hypochlorite solution for 1 minutes and then washed in sterilized distilled water for 3 times to remove traces of sodium hypochlorite. These pieces were then transferred aseptically to sterilized blotter paper to remove excess moisture and then to petri-plates containing PDA media inoculated petri plates were kept in incubator at 26 ^{0}C ± 2 ^{0}C and examined at frequent intervals to see the growth of fungus developing form different pieces. Early growing fungal mycelia was transferred to another PDA plats and allowed to grow for next seven days at 26±2 ^{0}C. These cultures were observed under microscope and the stock cultures were kept in refrigerator for further studies.

Collection of cow urine

Cow urine was collected from dairy cattle before milking in the morning at livestock research centre SVPUA&T, Meerut. The urine from the individual cows was mixed to avoid variations from individual cows. Cows from which urine was collected were not under any antibiotic treatment prior to or during the period of urine collection.

Antifungal activity of cow urine

The efficacy of fresh cow urine to inhibit test fungi was determined by poisoned food technique. PDA medium was amended with different concentrations of fresh cow urine *viz*., 5, 10and 15 sterilized by autoclaving and added to labelled petri plates. Fungal discs of 5 mm diameter were cut from the periphery of 5 days old culture of *Fusarium oxysporum* f. sp. *ciceris, Sclerotium rolfsii* and *Sclerotinia sclerotiorum* were inoculated aseptically on PDA plates poisoned with different concentrations of cow urine and potato dextrose agar medium without adding of cow urine served as control. The plates were incubated for 7 days at 26±2 ^{0}C. Colony diameters in mutual perpendicular directions were measured on the seventh day. The experiment was carried in triplicate and average colony diameter was recorded. Antifungal activity was recorded in terms of inhibition of mycelial growth (%) and calculated using the formula:

Inhibition of mycelial growth (%) = (C–T/C) X 100

Where,
C is average diameter of fungal colony in control plates and T is average diameter of fungal colony in poisoned plates (19, 20).

Result and Discussion

In the present studies three fungal pathogens *viz*, *Fusarium oxysporium* f. sp. *ciceris*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* were isolated from the diseased chickpea plant. Data presented in Table 1 depicts that all concentrations (5, 10, and 15%) of cow urine were effective which exhibited the significant inhibition in the growth of tested fungal plant pathogens. With increase in concentration of cow urine there was corresponding increase in the inhibition of mycelial growth of the fungal pathogens. The diameter of the fungal colonies in poisoned plates was lesser when compared to control plates and it indicates the antifungal effect of cow urine. 100% inhibition was shown against *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* was recorded in all the tested concentration after 144hours of inoculation (Figure 3&4), whereas maximum inhibition was recorded with 15 percent concentration of cow urine in *Fusarium oxysporum* (50.00%) after 192 hours of inoculation (Figure 1 & 2).

Table 1: Effect of different concentration of cow urine on mycelial growth inhibition of pathogens

Cow urine concentration	*Fusarium oxysporum* (192 Hrs.)		*Sclerotinia Sclerotiorum* (144 Hrs.)		*Sclerotium rolfsii* (144hr)	
	Mycelial growth of pathogen (mm)	Percent inhibition	Mycelial growth of pathogen (mm)	Percent inhibition	Mycelial growth of pathogen (mm)	Percent inhibition
5%	90.00	0.00	0.00	100	0.00	100
10%	78.33	12.96	0.00	100	0.00	100
15%	45.00	50.00	0.00	100	0.00	100
Control	90.00	-	90.00	-	89.33	-
C.D. (0.05)	1.35	-	1.55	-	1.12	-

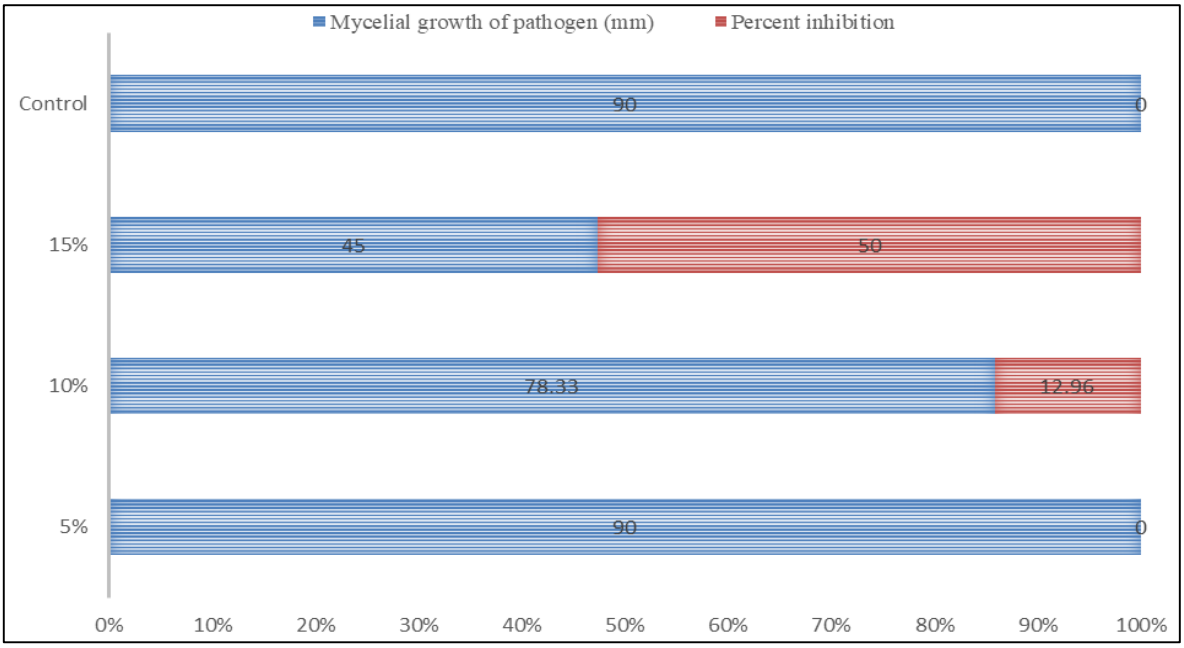

Fig 1: Effect of different concentration of cow urine on mycelial growth inhibition of *Fusarium oxysporum* f. sp. *ciceris*

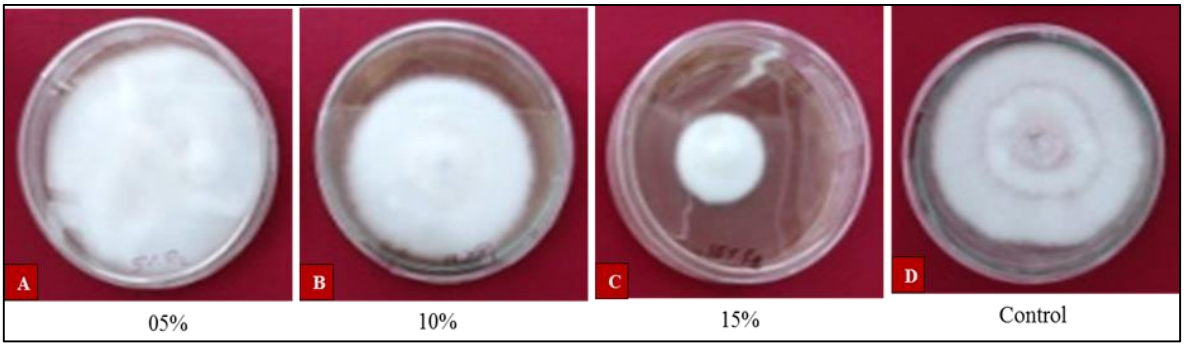

Plate 1: Effect of different concentrations of cow urine on *Fusarium oxysporum* f. sp. *ciceris*

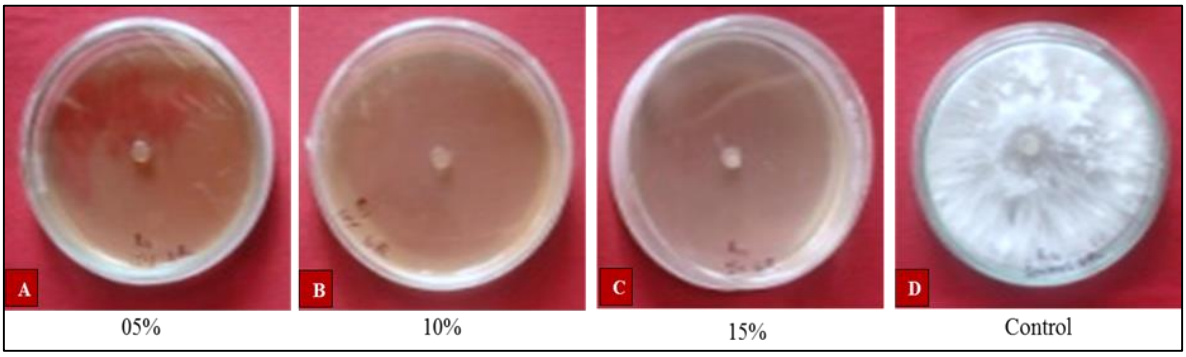

Plate 2: Effect of different concentrations of cow urine on *Sclerotium rolfsii*

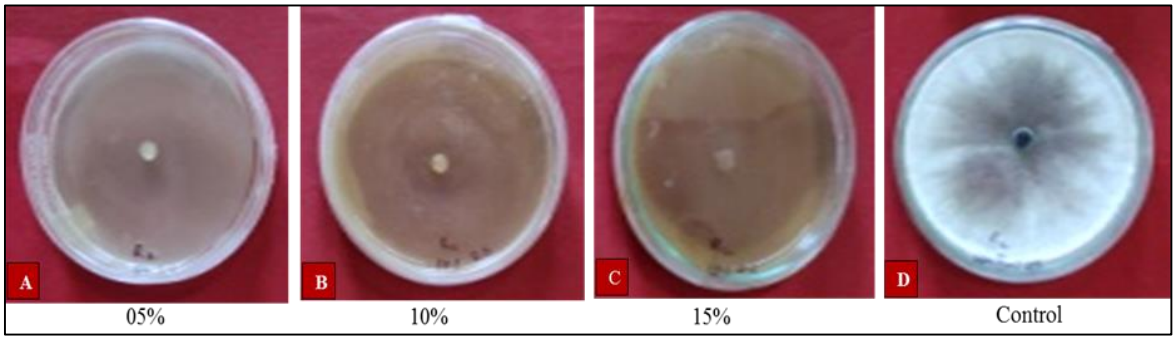

Plate 3: Effect of different concentrations of cow urine on *Sclerotinia sclerotiorum.*

Different authors have focused on different growth characteristics of different species of fungi as well as varying concentrations of cow urine. In some cases, cow urine has been used in combination with plant extracts or cow dung or as cow urine concentrate after distillation. In all cases, it has been proved that cow urine can be an effective treatment and control of fungal infections. The results have shown that, at varying concentrations of cow urine had considerable effect on the fungal growth of *Fusarium oxysporium* f. sp. *ciceris*, *Sclerotinia sclerotiorum* and *Sclerotium rolfsii*. It is clear from the results that all the concentration of cow urine showed maximum inhibition in growth of *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* pathogens as compared to control. Inhibitory activity of cow urine against fungal pathogens have been reported by different researchers Pandia *et al*. (2019) [16] found cow urine has most effective significant inhibitory effect against *A. alternata* with mycelial growth inhibition of 92.23 per cent at 7.5 per cent concentration. Basak *et al*. (2002) [4] showed the inhibitive activity of cow urine against *Sclerotinia sclerotiorum* causing sclerotinia rot in cucumber, *Fusarium solani* f. sp. *cucurbitae* causing root rot disease of cucumber. Akhter *et al.* (2006) [1] found the inhibitory efficacy of combination of cow urine with *Calotropis procera* against conidial germination of *Bipolaris sorokiniana*, causative agent of leaf blight of wheat. Murugan *et al.* (2012) [9] showed the efficacy of cow urine and cow urine with *Pongamia pinnata* seed against bacterial leaf blight of paddy caused by *Xanthomonas oryzae* Pv. *Oryzae*.

Conclusion

The present investigation revealed that, cow urine significantly inhibits the growth of all above three tested soil borne plant pathogens of chickpea. The use of cow urine is a cost effective and ecofriendly approach to control phytopathogenic fungi and better alternative to synthetic chemicals which are expensive and pose potential danger to the farmers, marketers, consumers, and environment. Further, field studies are to be carried out to justify the possible utilization of cow urine against soil borne plant pathogens of chickpea. Therefore, the cow urine can be used as bio pesticide for management of soil borne diseases.

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